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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No.: 37945-0018

Applicants(s):	Angus George DALGLEISH *et al.*	Confirmation No.: 3688	
Appl. No.:	09/857,691	Examiner: M. Davis	
Filing Date:	September 5, 2001	Group Art Unit: 1642	
Title:	USE OF HUMAN PROSTATE CELL LINES IN CANCER TREATMENT		

DECLARATION UNDER 37 C.F.R. § 1.132

I, Anthony I. Walker, a citizen of the United Kingdom with an address at 50 Hampstead Way, London NW11 7XX, United Kingdom ,do hereby declare as follows:

1. I am the Chief Executive Officer of Onyvax Limited, located at St. Georges Hospital Medical School, London SW17 0RE, United Kingdom, and a co-inventor of the captioned application.

2. I received my B.A. degree in Natural Science, with Honors, in 1981 and my Ph.D. in Biochemistry from the University of Cambridge in 1985. I have been engaged in research in the fields of natural sciences and biochemistry for over 10 years. I have held numerous scientific and executive positions for the past 10 years. A copy of my *curriculum vitae* is attached as Appendix A.

3. I have read and understood the Office Actions and cited references issued by the U.S. Examiner in the instant application, including the Non-Final Office Action dated August 1, 2003 (Paper No. 13). (*see* list of pending claims in Appendix B).

4. I provide this declaration in further explanation of additional experimental evidence and scientific literature provided herewith by Applicants in support of the instant application and in response to the Non-Final Office Action of August 1, 2003. I have read and understand the attached additional experimental data and scientific literature. (*see* additional experimental data in Appendix C).

5. The subject of the claimed invention is directed to the use of normal, non-malignant allogeneic cells derived from one or more non-cancerous prostate tissue samples that may be used as prostate cancer vaccine treatments. Allogeneic cells are cells that are taken from different individuals of the same species. Allogeneic cell lines disclosed in the instant specification include PNT2, NIH1542, LnCap, and Du145, of which PNT2 is derived from non-cancerous tissue. The claimed allogeneic cells are lethally irradiated to ensure that they are replication-incompetent prior to use in a human or mammal. The claimed invention overcomes problems associated with known cell-based cancer vaccines, because the claimed cell lines can be produced (sourced from tissue samples, immortalized and cultured *in vitro* indefinitely on a large scale) significantly more easily than cell lines derived from tumor tissue material (*see* page 3 of the instant specification).

6. The purpose of the attached additional experimental data is to provide evidence to support the principle of providing an allogeneic vaccine of the type recited in claim 1 of the instant application, to a cancer subject.

7. Experiments 3 and 4 contain new experimental data compared to the previous experiments disclosed in the instant specification. In experiments 3 and 4, normal mouse non-cancer prostate and renal cell lines were administered to mouse cancer subjects, as opposed to the data disclosed in the instant specification in which several different cell line combinations comprising metastatic, primary, and normal human prostate cell lines PNT2, NIH1542, LnCap, and Du145, were administered to human prostate cancer patients. (*see* Appendix C)

1) Experiment 1 (Phase I trial) involved treating human prostate cancer subjects with the human cell lines LnCap, NIH1542, and PNT2. The data and results from this experiment, disclosed in the instant specification, show that 50% of the treated patients mounted a T-cell proliferative response to at least one of the cell lines (*see* specification, p. 9 and Figure 1). Additionally, the serum prostate specific antigen (PSA) levels in the treated cancer patients dropped or partially stabilized, compared to the exponentially rising serum PSA levels normally found in cancer patients (*see* specification, p. 11). These results prove that the claimed combined prostate cell lines are effective vaccines against cancer.

2) Experiment 2 (Onyvax R&D #1) involved treating a mouse melanoma subject with a mouse melanocyte (derived from non-cancerous tissue) cell line. This experiment is intended to provide proof of the principle that different types of cell lines may be used in cancer vaccines to treat cancer and that the claimed invention is useful because it can be used to treat many different tumors, including melanoma.

3) Experiment 3 (Onyvax R&D #2) involved new data relating to treatment of a mouse prostate cancer subject with a proprietary non-cancerous mouse prostate cell line. Treatment of the cancerous mice with the non-cancerous mouse prostate cell line showed significant efficacy as a cancer vaccine. The purpose of this experiment is to prove the general principle that non-cancer prostate cell lines may be used to treat tumors. The results of this experiment also support the principle that normal, immortalized non-cancerous cell lines may be used as cancer vaccines to treat cancer.

4) Experiment 4 (Onyvax R&D #2) involved new data relating to the treatment of a mouse renal subject with a non-cancerous mouse renal cell line. The non-cancerous renal cell line showed significant efficacy as a cancer vaccine. The purpose of this experiment is to prove the general principle that normal cell lines other than prostate cell lines, such as renal cell lines, may be used as vaccines in cancer models.

Together, Experiments 2, 3 and 4 show, in relevant animal models, that non-cancer derived cell lines can treat three different types of cancer.

8. The following six references are provided in support of the specification of the instant application and the attached additional experimental data in Appendix B. These references also highlight the importance of the prostate-specific antigen velocity (PSAV) and the PSA doubling time (PSADT) as indicators of cancer in patients. The PSAV is the rate of change in PSA over time, while the PSADT is the time that it takes for the serum PSA levels to double (doubling time). Both PSADT and PSAV are well-known in the art as prognostic markers of prostate cancer.

9. Wu *et al.* (*Intl. J. Cancer*, 77(6): 887-94, 1998), also cited at page 11 of the Examiner's Non-Final Office Action of August 1, 2003, disclose that there is a correlation between increased serum PSA levels and disease progression of prostate cancer. After tumor inoculation, PSA serum levels are elevated (p. 890). The growth of such prostate tumors may be reliably monitored by serum PSA because of the production of PSA by LnCap cells (pp. 892-93). (*see* Appendix D).

10. Sartor *et al.* (*Int. J. Radiation Oncology Biol. Phys.*, Vol. 38, No. 5, pp. 941-947, 1997) disclose that rapidly rising PSA is the most powerful predictor of metatstatic failure (p. 945, col. 1). Sartor *et al.* assessed 2,667 PSA values from 400 patients who had undergone radical radiotherapy. Metastatic disease developed in 46% of the patients with a PSADT of less than 6 months, compared with 8% of those with a PSADT between 6 and 12 months (p=0.0002). Sartor *et al.* proved that PSA is a valuable tumor marker for prostate cancer because rising PSA levels following treatment of prostate cancer indicate eventual clinical failure (*i.e.,* disease progression and death) of a patient (p. 941, p. 943, col. 2, table 1). (*see* Appendix E).

11. D'Amico *et al.* (*J. Clin. Oncology*, Vol. 20, No. 20, pp 4576-4573, 2002) disclose that PSADT may be used as a predictor of time to prostate cancer-specific death (PCSD) following PSA failure in patients who have undergone radical radiotherapy (p. 4571, col. 2 and p. 4572, col. 2, last paragraph). At p. 4567, col. 2, D'Amico *et al.* propose that PSADT can be used as a surrogate for PCSD. Monitoring of serum PSA levels after treatment is commonly used for measuring prostate cancer (p. 4570, col. 2). PSADT is a

significant predictor of time to PCSD (p. 4571). PSA doubling times in the range of 0.5 to 1 year are considered dangerous, while PSA doubling times of about 10 years are considered benign. (*see* Appendix F).

12. Pound *et al.* (*JAMA*, Vol. 281, No. 17, pp. 1591-1597, 1999) disclose that PSADT is predictive of probability and time to development of metastatic disease (p. 1593, col. 2, middle paragraph, figure 3C, and p. 1597, col. 3 (conclusion)). The study in Pound *et al.* followed 1,997 men who had undergone radical prostatectomy. Fifteen percent of the men treated showed elevations in PSA levels, while 34% of the remaining men not treated with hormone therapy developed metastatic disease within the study period. PSADT ($p<0.001$) is predictive of the probability and time to the development of metatstatic disease (p. 1591). Shorter PSADTs (<6 months), were more indicative of disease when compared with local recurrence (p. 1596, col. 2). (*see* Appendix G).

13. Roberts *et al.* (*Mayo Clin. Proc.*, 76:576-581, 2001) disclose that PSADT is an important predictor of prostate cancer progression (pp. 579-80). Between 1987 and 1993 Roberts *et al.* examined prostate cancer progression in 2,809 men with elevated PSA levels, and the effect of PSADT on such disease progression (p. 576). Roberts *et al.* teach that a rising PSA level suggests evidence of residual or recurrent prostate cancer (p. 576). PSADT is an important predictor of systemic progression of prostate cancer, and is the "strongest" predictor of time to metatstatic disease because it can be used to predict how long a patient may remain free of metastasis after biochemically detected recurrence. A rising PSA level suggests evidence of residual or recurrent prostate cancer (pp. 580-81). (*see* Appendix H).

14. Vollmer *et al.* (Cancer, Vol. 83, No. 9, pp. 1989-1994, 1998) disclose that PSAV can be correlated with disease progression and survival time of cancer patients (p. 1989). Vollmer *et al.* studied the effect of megestrol acetate dosage on survival times of 148 men with hormone refractory prostate carcinoma (HRPC) and correlated a measurement of the average relative PSAV and response to cancer treatment. A reduction in PSAV over a sustained period in HRPC may translate into a clinical benefit because there is an inverse correlation between PSAV and survival time in HRPC. (*see* Appendix I).

15. Although the above-mentioned references focus on the utility of using PSA levels, including PSADT and PSAV, as predictors of disease progression and likely clinical failure (*i.e.,* death), the reverse principle could be applied to analyzing data of treatment of prostate cancer subjects by vaccination using cell lines. Accordingly, while increased rates of serum PSA production are associated with disease progression, a reduction in the rate of increase of serum PSA levels, including the PSADT, and/or PSAV, would be an efficacy indicator of a prostate cancer vaccine.

16. The above-cited references also support the data in the instant specification, specifically Figure 4, in which PSA levels (shown on the vertical axis in ng/ml) are shown over time (horizontal axis) in cancer patients. Figure 4 illustrates how, after the first time point when the claimed cell lines are administered, there is a drop or partial stabilization of the PSA values, which normally continues to rise in cancer patients, often exponentially. This data, in light of the above-cited references, indicates that decreases in PSAV, PSADT, or both, is an indication that prostate cancer cell growth is inhibited.

Furthermore, a reduced PSAV level is not necessarily the result of an anti-PSA antibody response with subsequent removal of the antibody/PSA complex. The cell culture data in the specification (*see* specification, pp. 9 and 11), as supported by the new experimental data and scientific references illustrates that the three claimed cell lines, PNT2, LnCaP, and NIH1542, are effective in treating prostate cancer because the serum prostate specific antigen (PSA) levels in the treated cancer patients dropped or partially stabilized, compared to the exponentially rising serum PSA levels normally found in cancer patients. Thus, despite the unpredictability of previous approaches towards cancer therapy, Applicants have shown through the experimental data in Appendix B that the claimed cell line combinations, when used in a vaccine, are effective in treating cancer and other tumors, such as melanoma. Furthermore, the results of the attached new experimental data, prove that other combinations of non-cancer derived cell lines may be effective cancer vaccines and would not constitute undue experimentation.

I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful false statements, and the like so made, are punishable by fine or imprisonment, or both, under § 1001, Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

1 Dec 2003
Date

Anthony Ian Walker, Ph.D.
Chief Executive Officer
Onyvax Limited

Anthony Walker, PhD – Curriculum Vitae

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London NW11 7XX

Tel: 0771 3402 771
E-mail: anthony@walker.net

Personal details: Born 11 October 1960, London, UK
Married, two children (born 1992 and 1995)

Career summary:

Prior to co-founding Onyvax Ltd. as Chief Executive in 1997, Anthony Walker spent ten years as a management consultant to the chemical, pharmaceutical and biotechnology industries. He had previously been employed by ICI (now Zeneca) in Berkshire and Brazil, contributing to some of that company's early forays into biotechnology. Anthony Walker holds an Honours degree in Natural Science (Physiology) and a Ph.D. in Biochemistry (Cell Physiology/Molecular Biology) from the University of Cambridge.

1997- present: Onyvax Ltd.

With co-founders Peter Smith, PhD and Prof. Angus Dalgleish, Anthony Walker formed Onyvax Ltd. to develop cell-based vaccines to treat cancer. At the time of its first business plan in March 1997, Onyvax had no intellectual property, no ongoing research programs and no employees – it was characterized as "three men and a business plan". Within an unsalaried period of 9 months, the team raised first round venture funding from 3i, Alta Berkeley, SR One and the PS Wallenberg Trust, with additional funding from the founders and the company's non-executive Chairman Barrie Haigh. Key achievements of Onyvax include:

- Raised £4.4m venture funding in 12/97 and second round funding of £10.5m in 11/00 (new investors were Merlin BioSciences and MB Ventures). Secured a £0.3m DTI LINK grant in 1998;
- Recruited, motivated and managed a team of 40 based at the company's premises at St. George's Hospital Medical School, London;
- Initiated a 60-patient clinical trial of Onyvax-P for prostate cancer within 12 months of closing the first funding round; initiated two clinical trials in colorectal cancer;
- Filed seven patent applications; created the world's largest bank of human prostate cell lines;
- In-licensed Onyvax-105 from Cancer Research Campaign Technology and in-licensed several cell lines from various sources at low single digit royalty shares;
- Secured a CTX for Phase II trials of Onyvax-P; recruited 50 patients within 14 months
- Formed research collaborations with SR Pharma plc; University of York; University of Rotterdam; Nottingham Trent University; University of Bochum;
- Evaluated over 100 business development opportunities; maintained a comprehensive competitor intelligence database, updated daily.

1997 – present: Other Appointm nts

June 2001 – October 2002 Non-executive director of Proteome Sciences plc Chairman of Remuneration Committee; member of Audit Committee. Resigned due to additional workload following management changes at Onyvax.

1998 – present Member of various BBSRC grant committees including: Genes & Developmental Biology; Functional Genomics; Small Business Research and Innovation.

1987 - 1997: Management Consulting

At Arthur D. Little (1987-94), Anthony Walker launched the UK Healthcare Practice and was appointed a European Director. Within 12 months of founding the Practice, for which he had full P&L responsibility, he had recruited a group of 10 professionals generating a profitable business volume in excess of $1m/y. In 1994, he was recruited by The Wilkerson Group in London to lead strategic consulting in the UK, Benelux and Scandinavia. His clients included seven of the (then) top 20 European pharmaceuticals groups and over 25 biotechnology companies.

During his ten year career as a management consultant, he specialized in due diligence, strategy development, R&D management and corporate development for the chemical, pharmaceutical and biotechnology industries. Representative projects and clients include:

Corporate development (M&A, due diligence, alliances):

- Conducted due diligence and prepared Expert's Reports for the flotation on the London Stock Exchange of Peptide Therapeutics plc, SkyePharma plc, PPL Therapeutics plc and the AIM listings of Alizyme plc and Oxford BioMedica plc;

- Directed several search and due diligence assignments for European clients seeking acquisition, strategic alliance and licensing opportunities in Europe and the US;

- Due diligence analysis for a large oncology/immunology pharmaceuticals acquisition. Dr. Walker was a member of the core due diligence team and led the analysis of the target company's R & D pipeline. He also contributed to the formulation of post acquisition integration plans;

- Supported a major Rx company and its investment bank in establishing a global strategic alliance, assessing the relative values of the contributions from each prospective party and developing a negotiating position for the client;

Technology assessment and R&D portfolio analyses:

- For a large European Rx company, analyzed the portfolio consisting of 50 projects (NCEs, new dosage forms, new clinical indications); together with a client task force, re-prioritized the portfolio and formulated a long range technology strategy;

- Developed the analytical approach and directed the activities of a team of technical experts in the evaluation of 25 R&D institutes in the former East Germany;

- Technology benchmarking analyses in several areas including drug delivery systems, biotechnology, R&D competitive intelligence;

Business organization and processes:

- For a mid-size Rx company, formulated project-based approaches aimed at reducing development lead times for NCE projects by 50%;
- Analysis of the role of the corporate centre of a large European pharmaceutical company, and development of recommendations to enhance the efficiency and effectiveness of corporate management;
- For a European OTC pharmaceuticals company, developed a project-based R&D structure and portfolio management system to overcome the limitations of the previous model which had been dominated by functional departments;
- Working with a client team, developed reporting structures, decision-making bodies and management processes to ensure coherence and clarity in the prioritization of the R&D portfolio;

Biotechnology:

- Dr. Walker was a member of a team advising the Dutch Ministry of Economic Affairs on policy for fostering the industrial application of biotechnology;
- Assessed entry opportunities in industrial biotechnology for Dow Europe;
- Developed a biotechnology strategy for a leading fine chemicals company;
- Strategy development for a large European company seeking to harness biotechnology as the basis for a new core business activity;

Business portfolio analysis and strategy development:

- For a European healthcare group, determined strategic priorities among its 15 business units in the light of their competitive positions and exposure to external threats;
- For one of the leading Japanese pharmaceutical companies, development of a long-range strategic plan for building its fledgling presence in Europe;
- Business unit and product strategy development for numerous clients, spanning a broad range of therapeutic areas, including oncology, auto-immunity, ophthalmics, urology, gastro-intestinal, anti-infectives, psychiatry and OTC products;
- On behalf of a top 5 global Rx company, characterized the changing customer base in Europe, focusing on emerging stakeholders in the purchasing, prescribing and regulatory processes and assessing the implications of European health care reform;

1985 - 1986: ICI (subsequently known as Zeneca)

Recruited to a management development scheme, with an initial two year period rotating through various functions prior to a managerial placement. The first eight months were spent as an Experimental Officer at Jealott's Hill Research Centre, working on the novel growth regulator paclobutrazol (PP333). This was followed by a six month secondment to Brasil to conduct the development of paclobutrazol, a novel growth regulator, in a range of agricultural and horticultural applications. On his return to the UK, he was attached to the group responsible for strategy development across a range of opportunities for ICI in biotechnology. At the end of the two year period, Anthony Walker was offered a five-year commercial management assignment in Francophone West Africa. He declined in favour of joining Arthur D. Little in London.

Education:

1981-84:	PhD in Biochemistry, University of Cambridge Supervisor: Tim Hunt, PhD (Nobel Laureate 2001) President, Emmanuel College Middle Combination Room Bachelor Scholar, Emmanuel College
1978-81:	First class BA (Natural Sciences), University of Cambridge Senior Scholar, Emmanuel College (1979, 1980)
1968-78:	University College School, Hampstead, London

Pending Claims:

Claim 1. (previously presented) An allogeneic immunotherapeutic agent for the treatment of prostate cancer comprising three human prostate cell lines from three different sources, of which one, two or three cell lines are derived from normal tissue(s), wherein each said normal tissue(s) is (are) from a source which is a non-cancerous prostate.

Claims 2-10. (withdrawn)

Claim 11. (previously presented) An allogeneic immunogenic composition comprising an immunotherapeutic agent of claim 1 combined with a vaccine adjuvant selected from the group consisting of BCG, *M. Vaccae*, Tetanus toxoid, Diphtheria toxoid, *Bordetella Pertussis*, interleukin 2, interleukin 12, interleukin 4, interleukin 7, Complete Freund's Adjuvant, Incomplete Freund's Adjuvant, and non-specific adjuvants.

Claim 12. (previously presented) An immunogenic composition comprising an immunotherapeutic agent of claim 1 combined with a vaccine adjuvant, wherein the adjuvant is a mycobacterial preparation.

Claims 13-22. (withdrawn)

APPENDIX C

Experiment	Subjects	Vaccine Cell Lines Used to Treat Subjects	Results & Conclusions	Corresponding Claims
1. Phase I trial	Human with prostate cancer	LnCaP (metatstatic human prostate cell line) NIH1542 (primary human prostate cell line) PNT2 (normal human prostate cell line)	Data and results are disclosed in the instant application.	claims 1, 2, and 8 of PCT/GB99/04129 (published 6/15/00)
2. Onyvax R&D #1	Mouse with melanoma	Mouse, non-cancer	Data and results are disclosed in the instant application. This experiment uses a different species of cancer and different types of cell lines relative to the claimed combination. The efficacy of the results of this experiment illustrates the general principle of using an immortalized normal cell line as a cancer immunotherapeutic, but is not intended to support the claimed types of cell lines.	Corresponds partially to claim 3 of PCT/GB99/04129 (published 6/15/00), but is not human or directed to prostate cancer.

APPENDIX C

Experiment	Subjects	Vaccine Cell Lines Used to Treat Subjects	Results & Conclusions	Corresponding Claims
3. Onyvax R&D #2	Mouse prostate	Mouse, non-cancerous prostate	This is new data. We have developed our own mouse prostate cancer model whereby we used a proprietary (non-deposited) mouse normal prostate cell line as the vaccine. The challenge was with a previously described tumourigenic mouse prostate cell line (RM9). We have data showing significant efficacy of the normal cell line as an immunotherapy. This experiment uses different types of cell lines relative to the claimed combination, although it is the same type of cancer (prostate). These results support the general principle that immortalized normal cell lines are useful as cancer vaccines. These results also show that a mouse melanoma model supports a claim in prostate cancer.	partially relates to claim 3 of the instant application, but is not human.

APPENDIX C

Experiment	Subjects	Vaccine Cell Lines Used to Treat Subjects	Results & Conclusions	Corresponding Claims
4. Onyvax R&D #3	Mouse renal	Mouse, non-cancerous renal	This is new data. We have developed our own model of mouse renal cancer whereby we used a proprietary (non-deposited mouse normal renal cell line as the vaccine. The challenge was with a previously described tumourigenic mouse renal cell line. (RenCa). We have data showing significant efficacy of the normal cell line as an immunotherapy. The results of using this variant cancer species and different types of cell lines relative to the claimed combination supports the general principle that immortalized normal cell lines are useful as cancer vaccines.	

Int. J. Cancer: 77, 887–894 (1998)

Publication of the International Union Against Cancer
Publication de l'Union Internationale Contre le Cancer

ESTABLISHING HUMAN PROSTATE CANCER CELL XENOGRAFTS IN BONE: INDUCTION OF OSTEOBLASTIC REACTION BY PROSTATE-SPECIFIC ANTIGEN-PRODUCING TUMORS IN ATHYMIC AND SCID/bg MICE USING LNCaP AND LINEAGE-DERIVED METASTATIC SUBLINES

Tony T. WU[1], Robert A. SIKES[1], Quanjun CUI[2], George N. THALMANN[4], Chinghai KAO[1], Cheryl F. MURPHY[3], Hua YANG[1], Haiyen E. ZHAU[1], Gary BALIAN[2] and Leland W.K. CHUNG[1]*

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LNCaP lineage-derived human prostate cancer cell lines C4-2 and C4-2B$_4$ acquire androgen independence and osseous metastatic potential *in vivo*. Using C4-2 and C4-2B$_4$ the goals of the current investigation were 1) to establish an ideal bone xenograft model for prostate cancer cells in intact athymic or SCID/bg mice using an intraosseous route of tumor cell administration and 2) to compare prostate cancer metastasis by administering cells either through intravenous (i.v.) or intracardiac administration in athymic or SCID/bg mice. Subsequent to tumor cell administration, prostate cancer growth in the skeleton was assessed by radiographic bone density, serum prostate-specific antigen (PSA) levels, presence of hematogenous prostate cancer cells and histopathologic evaluation of tumor specimens in the lymph node and skeleton. Our results show that whereas LNCaP cells injected intracardially failed to develop metastasis, C4-2 cells injected similarly had the highest metastatic capability in SCID/bg mice. Retroperitoneal and mediastinal lymph node metastases were noted in 3/7 animals, whereas 2/7 animals developed osteoblastic spine metastases. Intracardiac injection of C4-2 in athymic hosts produced spinal metastases in 1/5 animals at 8–12 weeks post-injection; PC-3 injected intracardially also metastasized to the bone but yielded osteolytic responses. Intravenous injection of either LNCaP or C4-2 failed to establish tumor colonies. Intrailiac injection of C4-2 but not LNCaP nor C4-2B$_4$ cells in athymic mice established rapidly growing tumors in 4/8 animals at 2–7 weeks after inoculation. Intrafemoral injection of C4-2 (9/16) and C4-2B$_4$ (5/18) but not LNCaP (0/13) cells resulted in the development of osteoblastic bone lesions in athymic mice (mean: 6 weeks, range: 3–12 weeks). In SCID/bg mice, intrafemoral injection of LNCaP (6/8), C4-2 (8/8) and C4-2B$_4$ (8/8) cells formed PSA-producing, osteoblastic tumors in the bone marrow space within 3–5 weeks after tumor cell inoculation. A stepwise increase of serum PSA was detected in all animals. Reverse transcription-polymerase chain reaction (RT-PCR) to detect hematogenously disseminated prostate cancer cells could not be correlated to either serum PSA level or histological evidence of tumor cells in the marrow space. We have thus established a PSA-producing and osteoblastic human prostate cancer xenograft model in mice. *Int. J. Cancer* 77:887–894, 1998.
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Adenocarcinoma of the prostate has been recognized consistently as the leading cause of cancer and the second leading cause of cancer death in North American men (Boring *et al.*, 1994). According to 1996 American Cancer Society statistics, the newly diagnosed cases of prostate cancer have risen to 334,500 in the United States, and prostate cancer is the most commonly diagnosed cancer and the second leading cause of cancer deaths in men (Parker *et al.*, 1997). Because of increasing public awareness and screening programs, most of the recently diagnosed prostate cancer patients are clinical stage T1c or organ-confined (Catalona *et al.*, 1991, 1993; Stormont *et al.*, 1993). However, a certain percentage of patients present with disease beyond the confines of the prostate (Catalona *et al.*, 1991). Among these cases, disease progression to distant metastasis is almost unavoidable despite local or systemic therapies (Scher and Chung, 1994). Of those that metastasize, the bone is the most common and debilitating site (Scher and Chung, 1994; Stamey and McNeal, 1992).

Prostate cancer is known to grow and induce osteoblastic as well as osteolytic responses when it harbors at the bone marrow space (Frank, 1997). A survey of the literature shows that several human prostate cancer cell lines when injected subcutaneously (s.c.) (Thalmann *et al.*, 1994), orthotopically (Thalmann *et al.*, 1994), intravenously (i.v.) with/without inferior vena cava occlusion (Shevrin *et al.*, 1988; Wang and Stearns, 1991) or intraosseously (Berlin *et al.*, 1993; Soos *et al.*, 1996) induce skeletal metastases or intraosseous tumor growth. A fresh human prostate cancer xenograft, when implanted s.c. in SCID mice, has developed micrometastases in the bone marrow as detected by reverse transcription-polymerase chain reaction (RT-PCR) (Klein *et al.*, 1997). The LNCaP human prostate cancer model described by Thalmann *et al.* (1994) closely mimics the clinical prostate cancer progression in which a serum marker, prostate-specific antigen (PSA), was elaborated and the tumors progress from androgen dependence to androgen independence with substantial osteoblastic reactions associated with skeletal metastases. Despite the availability of previously described human prostate cancer metastatic models (Klein *et al.*, 1997; Thalmann *et al.*, 1994), there remain significant limitations of each model system. The LNCaP human prostate cancer model (Thalmann *et al.*, 1994) is limited by a lengthy latent period between injection and detectable osseous metastasis. Intravenous and intraosseous models of PC-3 tumor growth and bone metastasis in immunodeficient animals generate exclusively osteolytic responses without reliable means to evaluate tumor volume using serum markers (Shevrin *et al.*, 1988; Wang and Stearns, 1991). While the primary tissue xenograft model results in PSA-producing tumors (Klein *et al.*, 1997), the extent of metastasis is limited and there is no evidence of metastasis-related osteoblastic reaction.

To improve human prostate cancer metastatic models, this study and others (Pettaway *et al.*, 1996; Sato *et al.*, 1997) used athymic and/or SCID/bg mice as hosts as well as to study interactions

Grant sponsor: National Institutes of Health; Grant numbers: CA-63341, CA-64863 and DK-47596.

Tony T. Wu and Robert A. Sikes contributed equally to this work.

*Correspondence to: Molecular Urology and Therapeutics Program, Department of Urology, Box 422, University of Virginia Health Sciences Center, Charlottesville, VA 22908, USA.

Received 14 January 1998; Revised 3 March 1998

between prostate cancer cells and their microenvironment. We have examined prostate cancer cells with a range of metastatic potential and evaluated the effects of different routes of tumor cell administration. We have established a protocol for rapid intraosseous prostate cancer lesions that should allow the direct study of cellular interactions between prostate cancer cells and bone stroma *in vivo* as well as provide a valuable screening tool for prostate cancer-directed adjuvant therapy.

MATERIAL AND METHODS

Animals, maintenance and monitoring

Thirty to 60-day-old male athymic mice (*nu/nu* strain, Harlan Sprague-Dawley, Indianapolis, IN) and male SCID/bg mice (University of Virginia Cancer Center breeding colony) were used for all *in vivo* experiments. Animals were housed under pathogen-free conditions in accordance with established NIH guidelines using an animal protocol approved by the University of Virginia Health Sciences Animal Research Committee. All manipulations with the animals were performed in a laminar flow hood with sterile techniques under methoxyflurane (Metofane, Pitman-Moore, Mundelein, IL) inhalant anesthetic unless noted otherwise. Experimental endpoints included elevation of serum PSA (>2 ng/ml), palpable tumor mass and/or the development of paraplegia. Animals were also sacrificed in the event of a loss of more than 50% of body weight or at the end of a 4-month observation period.

Cell lines and cultures

Cells and sublines. LNCaP, an androgen-responsive human prostate cancer cell line originally derived from supraclavicular lymph node with prostate cancer metastasis (Horoszewicz *et al.*, 1980, 1983), was obtained from Dr. G. Miller (University of Colorado, Denver); passages 26–28 and 46–48 were used for this study. C4-2 (passages 16–18), an androgen-unresponsive and metastatic subline, was derived from the parental LNCaP cells (Thalmann *et al.*, 1994; Wu *et al.*, 1994). C4-2B_4, a C4-2 derivative, was isolated from metastatic prostate cancer lesions found in the lumbar spine of an athymic murine host. Both cytogenetic (Thalmann *et al.*, 1994; Wu *et al.*, 1994) and comparative genomic hybridization (Hyytinen *et al.*, 1997) analyses showed that C4-2 and C4-2B_4 were of human origin and shared common marker chromosomes and chromosomal regions with the parental LNCaP cell line. C4-2B_4 was chosen over other bone metastatic sublines due to its aggressive metastatic potential when inoculated s.c. (data not shown). This subline was selected further by hygromycin (Calbiochem-Novabiochem, San Diego, CA) after transfection with plasmid pLXSH (obtained from Dr. D. Miller, University of Washington, Seattle) containing the hygromycin-resistance gene (Miller and Rosman, 1989). PC-3, an androgen-independent human prostate cancer cell line (Kaighn *et al.*, 1979) derived from a bone metastasis specimen, was purchased from the ATCC (Rockville, MD).

All cell lines were grown in T-medium [80% Dulbecco's modified Eagle's medium (GIBCO, Grand Island, NY), 20% F12K (Irving Scientific, Santa Ana, CA), 3 g/l $NaHCO_3$, 100 units/l penicillin G, 100 μg/ml streptomycin, 5 μg/ml insulin, 13.6 pg/ml triiodothyronine, 5 μg/ml transferrin, 0.25 μg/ml biotin, 25 μg/ml adenine], supplemented with 5% fetal bovine serum (FBS), until 80–90% confluent (Chang and Chung, 1989). The cells were tested and found to be consistently free of Mycoplasma.

Route of tumor cell administration

Intravenous injection. LNCaP or C4-2 cells (1×10^6) were administered via the tail vein into male athymic mice. Cells were suspended in phosphate-buffered saline (PBS) and were administered by a TB syringe with a 27g needle at 100 μl per mouse. A total of 9 animals were studied per cell line to assess tumorigenic and metastatic potential.

Intracardiac injection. Cells were prepared from subconfluent cultures as described by Sasaki *et al.* (1995). Anesthetized animals were placed in a supine position for tumor cell injection. LNCaP or C4-2 cells (5×10^5 cells in 50 μl PBS) were administered into the left ventricle of 5 male athymic mice each using a TB syringe attached with a 27g needle. The appearance of fresh arterial blood in the syringe marked the successful penetration of the ventricular wall. The same procedures were adopted for the intracardial administration of LNCaP (5 mice), C4-2 (7 mice) or PC-3 (3 mice) cells in male SCID/bg mice.

Subcutaneous injection. LNCaP, C4-2 or C4-2B_4 cells (2×10^7 cells/ml) were resuspended in T-medium plus 5% FBS and were injected at 2–4 sites at 100 μl/site as described previously (Thalmann *et al.*, 1994).

Pericranial administration. LNCaP, C4-2 or C4-2B_4 cells (5×10^5 cells in 50 μl PBS) were injected into the pericranial space using 3 male athymic mice for each study group. Briefly, a 19g needle was inserted through the scalp of anesthetized mice to scratch the periosteum of cranium. With the needle kept in place, cells were instilled to the scratched area using a calibrated Hamilton syringe (Reno, NV) inserted through the 19g needle. The puncture hole was compressed for several minutes to prevent backflow of the cell suspension.

Intrailiac injection. Anesthetized mice were placed in the prone position; the left hind leg was held firmly and kept in an abducted position. A TB syringe with attached 27g needle was inserted through the skin at a point 5 mm lateral to the lumbar spine and 5 mm below the iliac crest. The needle was guided toward the iliac crest and advanced with gentle drilling motion until the superficial cortex of iliac bone was penetrated. Human prostate cancer cells (1×10^6) resuspended in 50 μl of T-medium supplemented with 10% FBS were gently injected into the marrow space of iliac bone. Four male athymic mice each were inoculated with LNCaP and C4-2B_4 and 8 mice were injected with C4-2 cells.

Intrafemoral injection. Mice were anesthetized by intramuscular injection of a mixture of ketamine (50 mg/kg) and xylocaine (5 mg/kg). An incision was made in anesthetized mice along the right knee, and the patellar tendon and muscle were split longitudinally to expose the distal femur. A surgical scalpel (number 11) was used to drill a tiny hole on the cortex and cells were injected into the bone marrow space through the hole with a 27g needle. Cancer cells, resuspended in 50 μl PBS or culture medium, were introduced into the marrow space slowly to avoid extravasation. The needle was removed and the hole was sealed with bone wax, the patellar tendon reapproximated and the wound sutured.

Four prostate cancer cell lines (LNCaP, C4-2, C4-2B_4 or PC-3) were injected individually by the intrafemoral route to either athymic nude or SCID/bg mice with 5×10^5 or 1×10^6 cells each. The total number of athymic mice injected was 13, 16, 18 and 4, respectively. For SCID/bg, C4-2 or C4-2B_4 cells (1×10^6) were administered individually to 8 mice each for this comparative study. Cells were resuspended in either PBS or T-medium plus 10% FBS prior to administration. All SCID/bg mice were sacrificed at the end of 9 weeks.

PSA assay

Blood specimens (approximately 100 μl) were obtained from the tail vein at the intervals indicated for PSA assay. The sampled blood was centrifuged and the sera were stored at −20°C until assayed. PSA was determined by microparticle enzyme-linked immunosorbent assay (MEIA) using the Abbott IM_X machine and PSA assays (generously sponsored by Abbott Laboratories, Abbott Park, IL).

Bone X-ray and technetium (Tc) bone scans

All mice were imaged by X-ray using a Hewlett-Packard (Palo Alto, CA) Model 43804N X-ray system at 26 V for 40 sec immediately following sacrifice by CO_2 asphyxiation.

In the hope of detecting early skeletal lesions, all intracardially injected and 5 mice each (except PC-3) of the intrafemorally injected athymic mice were examined by radioisotopic bone scan

in the 5th, 7th, 9th and 11th weeks, essentially as described previously (Thalmann *et al.*, 1994). Animals were anesthetized by intramuscular injection of ketamine/xylocaine mixture. ^{99m}Tc-MDP (2.5 mCi) was injected through the tail vein. Three to 4 hr were allowed for the clearance of radioisotope from the kidney. The image acquisition time was approximately 10–20 min. Mouse bladders were emptied as much as possible to avoid the appearance of a hot spot in the urinary bladder.

RT-PCR detection of circulating tumor cells

In selected experimental groups, we conducted a nested RT-PCR analysis to detect PSA mRNA from circulating PSA-producing cells. We examined the possible correlation between tumor growth in the bone, serum PSA level and the presence of circulating tumor cells in the blood.

Eighty to 100 µl of blood was obtained by tail vein incision, using heparinized hematocrit tubes, from intraosseously injected SCID/bg mice 3, 5 and 9 weeks after tumor cell inoculation. Blood for RT-PCR analysis was processed essentially as described by Sato *et al.* (1997). In brief, blood samples were centrifuged and the blood cells were transferred to Eppendorf tubes. The red blood cells were hemolyzed by the addition of at least 5 vol of hemolysis buffer (0.15 M NH_4Cl; 0.01 M $NaHCO_3$; 0.09 mM EDTA). The cells were vortexed and incubated at room temperature for 10 min. The nucleated cells were collected by centrifugation at 1,750*g* for 3 min and the supernatant was carefully decanted. RNA isolation from the nucleated cells was done using 250 µl of UltraSpec RNA (BiotecX, Houston, TX) according to the manufacturer's recommendations. The RNA concentrations were determined by absorbance at 260 nm with a DU-650 spectrophotometer (Beckman, Schaumburg, IL). Five micrograms of total RNA was reverse transcribed into single-stranded cDNAs (ss-cDNA) for use in nested RT-PCR as described previously by Sikes and Chung (1992).

Nested RT-PCR was performed essentially as described by Israeli *et al.* (1994, 1995) using their published primer sequences. The PCR primers were synthesized by Genosys (Woodlands, TX). The external primer set produces a band of 486 bp, while the inner primer set produces a product of 355 bp from reverse transcribed RNA. These primer sets span an intron so that genomic DNA contamination can be excluded. The individual primer sets were optimized using Stratagene PCR optimization kit (La Jolla, CA). Buffer 10 [10 mM Tris-HCl (pH 9.2), 15 mM $MgCl_2$, 750 mM KCl] with 5% dimethyl sulfoxide (DMSO) was found to be the most efficient for both sets of primers.

Two microliters (10%) of ss-cDNA was used as the template for the outer primer PCR. The reaction was run for 25 cycles in a Perkin-Elmer GeneAmp PCR system 9600 (Norwalk, CT) under the following conditions: 94°C for 30 sec, 60°C for 30 sec and 72°C for 45 sec, followed by a final extension at 72°C for 7 min. Five microliters (10%) of the external PCR product served as the template for the internal PCR under the same reaction conditions for another 25 cycles. Five microliters of the final reaction product (10%) was analyzed by electrophoresis on 2% NuSieve/SeaKem 3:1 (FMC, Rockland, ME) agarose gel in 0.5 × TAE electrophoresis buffer. PCR products were visualized by ultraviolet (UV) illumination of ethidium bromide-stained gels.

Histopathological examination

Complete autopsies were done under a dissecting microscope on all animals. Any suspicious lesions and all intraosseous injected areas were removed and fixed in 10% formalin. All bone specimens were decalcified extensively with 0.25 M EDTA solution prior to paraffin embedding. Hematoxylin-eosin (H&E) staining was performed routinely for all sections.

For immunohistochemical staining, the tissue sections were deparaffinized with xylene, rehydrated with ethanol and treated with 3% H_2O_2 in methanol. Samples were blocked with Super-Block (Scytek, Logan, UT) and incubated with a monoclonal anti-PSA antibody (BioGenex, San Ramon, CA) at a 1:60 dilution for 45 min at room temperature. The specimens were rinsed with a 1:10 dilution of SuperBlock to remove excess antibody and were reacted subsequently with biotinylated anti-immunoglobulins for mouse, rabbit, guinea pig and rat antibodies (Multilink, BioGenex). After thorough rinse with a 1:10 dilution of SuperBlock to remove excess biotinylated antibodies, the tissue specimens were reacted with peroxidase-conjugated streptavidin (Label, BioGenex), rinsed and incubated with AEC chromagen (BioGenex) for color development. Immunostaining was visualized after couterstaining with hematoxylin.

Biostatistics

Analysis was performed using standard repeated measures models to compare rates of growth by mouse type and cell type, adjusting for the correlation between PSA measurements taken from the same animal. Statistical significance of the data sets was assessed using *F*-tests. The endpoint was ln(PSA + 1). One was added to allow for the natural logarithm transformation of the data. Mouse type (group) and cell type (type) were assigned to groups. Analysis of variance indicated a significant group effect (SCID/bg *vs.* athymic $p < 0.001$) and a significant type effect (cell line PSAs in SCID/bg *vs.* athymic $p < 0.001$).

RESULTS

Comparison of metastatic capability of LNCaP, C4-2 or PC-3 cells injected by either i.v. or intracardiac route

Table I shows that athymic mice failed to develop any metastasis when LNCaP or C4-2 cells were administered i.v. Neither gross lesions nor elevation of serum PSA was noted in these animals during a 4-month observation period. In contrast, when LNCaP or C4-2 cells were administered by intracardiac route in athymic hosts, C4-2 cells (but not LNCaP) presented with a paraspinal tumor mass that apparently (Fig. 1*a*) had expanded outwardly from a spinal micrometastasis (1/5, 20%) (Fig. 1*b*, arrow). The spinal metastasis showed little new bone formation but did show some remodeling near the hyperproliferative zone of the vertebral cartilage (Fig. 1*b*, arrowhead). The animal with spinal metastasis presented with a serum PSA level of 92 ng/ml at 6 weeks after tumor cell injection.

Because the intracardiac route yielded bony metastasis in athymic hosts, the metastatic potentials of LNCaP and C4-2 cells were tested in SCID/bg in the hope of developing an ideal murine model of human prostate cancer osseous metastasis, *i.e.*, a higher rate of prostate tumor development in the bone with a shorter latency period. Table I shows that C4-2 cells had higher frequency

TABLE I COMPARISON OF THE METASTATIC POTENTIAL OF EITHER I.V. OR INTRACARDIAC INJECTION OF HUMAN PROSTATE CANCER CELLS IN ATHYMIC OR SCID/bg MICE[1]

Cell lines	Route of injection	Number of mice	Metastatic lesions				
			Spine	Limbs	Lymph node	Adrenal	Others
Athymic Mice							
LNCaP	i.v.[2]	9	0	0	0	0	
C4-2	i.v.	9	0	0	0	0	
LNCaP	i.c.[3]	5	0	0	0	0	
C4-2	i.c.	5	1	0	0	0	1[4] (paraspinal soft tissue)
SCID/bg Mice							
LNCaP	i.c.	5	0	0	0	0	
C4-2	i.c.	7	2[5]	0	3	0	1 (paraspinal soft tissue)
PC-3	i.c.	3	0	2	0	1	2 (mandible)

[1]Animals were observed for 4 months.–[2]Intravenous injection through the tail vein with 1×10^6 cells resuspended in 50 µl of T-medium plus 10% FBS.–[3]Intracardiac injection of 5×10^5 cells resuspended in 50 µl of PBS.–[4]Same animal with spine metastasis.–[5]Both of them had lymph node metastases.

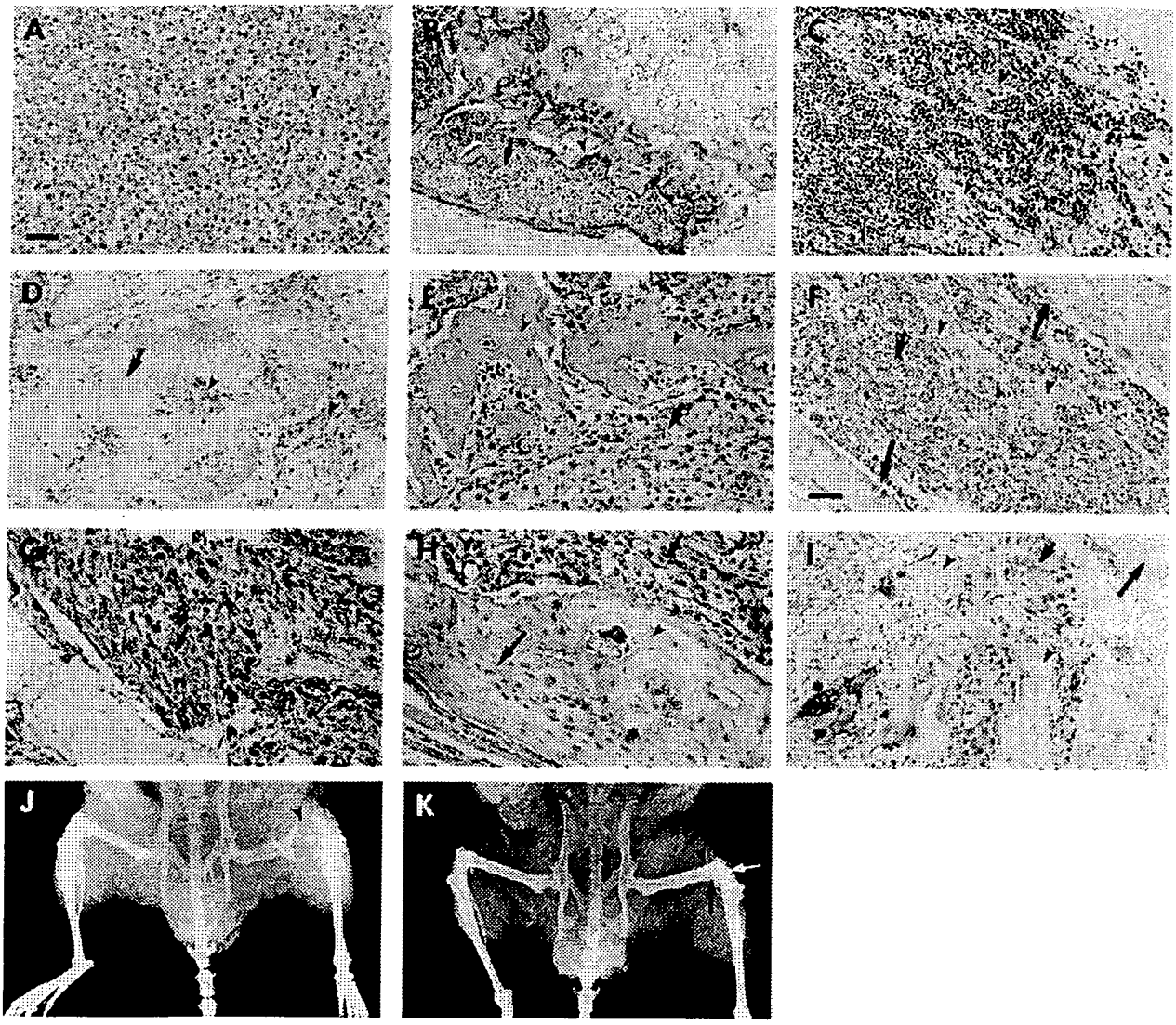

FIGURE 1 – H&E-stained tumor sections. *(a)* Paraspinal tumor mass excised from a SCID/bg mouse injected intracardially with C4-2 cells. Arrowhead indicates a representative mitogenic figure of which there are many in this tumor. *(b)* Micrometastasis to the spine from a SCID/bg mouse injected intracardially with C4-2 cells. Arrow indicates the carcinomatous micrometastasis within the vertebral body. Arrowhead indicates an area of bone remodeling in association with the tumor mass. *(c)* Lymph node with carcinoma infiltration (arrowheads). *(d)* Vertebral metastasis from an athymic murine host injected s.c. with C4-2 cells. The vertebral body is completely replaced by new osteoid (arrow) and poorly differentiated carcinoma in the process of new bone deposition (arrowheads). *(e)* Iliac tumor derived from the injection of C4-2 cells into athymic mouse iliac bone. Poorly differentiated carcinoma (arrow) and regions of new osteoid deposition (arrowheads) are shown. *(f)* Athymic mouse femur injected with C4-2 cells. Regions of new osteoid deposition or osteoblastic reaction (arrowheads) as well as regions of osteolytic activity (arrows) are clearly visible. *(g)* Immunohistochemical staining with a PSA antibody of tumor derived from the intrafemoral injection of C4-2 cells into athymic host. The pattern of PSA staining was variable and ranged from weak (arrow) to strong (arrowhead). *(h)* SCID/bg host injected intrafemorally with C4-2 cells. Clear presence of carcinoma indicated by short arrow. Areas of new osteoid are apparent (arrowhead) adjacent to regions of established osteoid (long arrow). A fissure (asterisks) is visible between the areas of new and old osteoid material. *(i)* Intrafemoral tumor derived from the injection of C4-2B_4 cells. Clear presence of carcinoma indicated by short arrow. Areas of new osteoid are apparent (arrowheads) adjacent to regions of established osteoid (long arrow). Regions of chondrocytic differentiation (asterisk) are also apparent in these tumor-bearing femurs. Additionally, C4-2B_4-derived tumors appear to have more blood vessels than other intrafemoral tumors. *(j)* X-ray of mouse femur injected with PC-3 cells. Bone resorption is clearly evident (arrowhead). *(k)* X-ray of mouse femur injected with C4-2 cells. Areas of osteoblastic reaction (arrowheads) are indicated by cortical thickening, while cortical thinning indicates regions of osteolytic activity (arrows). Scale bars: 50 μm *(a–e,g–i)*; 25 μm *(f)*.

of metastasis in SCID/bg mice compared to athymic mice, whereas none of the LNCaP cell injected mice developed distant metastasis. Three of 7 mice (43%) injected with C4-2 cells developed retroperitoneal (n = 2) and mediastinal lymphadenopathy (n = 1) (Fig. 1*c*) with a modestly elevated serum PSA (1.6–4.0 ng/ml) detected at 8–12 weeks after tumor inoculation. Among these, 2 animals had microscopic metastatic lesions found in the spine (data not shown). In comparison with the osteoblastic reaction (Fig. 1*d*,

arrow) of the poorly differentiated carcinoma (Fig. 1*d*, arrowhead) of the spinal metastasis induced by s.c. injected C4-2 cells, the animal injected intracardially with C4-2 had less osteoblastic reaction (Fig. 1*b*, arrowhead). One animal had a paraspinal tumor growth in the posterior cervical area without involving the vertebral body; serum PSA remained undetectable until the animal was sacrificed. By contrast, PC-3 cell injected mice had distant metastases, including the mandible (2/3), femurs (2/3) and adrenal glands (1/3) without detectable serum PSA (Table I). Unlike PC-3 cells, C4-2 cells appear to have acquired the unique, albeit low, potential to metastasize preferentially to the spine and lymph nodes from many different routes of injection.

Prostate cancer cell growth in athymic and SCID/bg mice by intraosseous or periosseous administration

To develop a model for establishing rapid prostate cancer growth in the bone, the direct administration of prostate cancer cells by pericranial, intrailiac and intrafemoral routes was compared. Adult male athymic mice injected with either LNCaP, C4-2 or C4-2B_4 cells by a pericranial route failed to develop tumors in a 4-month observation period. None of the 9 mice injected had detectable serum PSA.

Intrailiac administration of C4-2 cells resulted in tumors in 4/8 (50%) athymic mice injected with the development of paraplegia at 2–7 weeks (median: 4) after inoculation. Serum PSA was detected in 3/7 animals and ranged from 0.5 to 4.7 ng/ml. One animal developed paraplegia and died 2 weeks after inoculation before serum PSA could be evaluated. Histopathological examination disclosed poorly differentiated intramarrow tumor growth (Fig. 1*e*, arrow) with new bone formation (Fig. 1*e*, arrowhead). None of the animals which were injected with either LNCaP (0/4) and C4-2B_4 (0/4) cells intrailiacally established intraosseous growth or detectable serum PSA levels (Table II).

Intrafemoral injection into athymic mice resulted in the following tumor "take" rates: PC-3 (4/4, 100%) > C4-2 (7/9, 78%) > C4-2B_4 (5/11, 45%) > LNCaP (0/8, 0%). C4-2 and C4-2B_4, but not the parental LNCaP cells when injected intrafemorally, formed PSA-secreting tumors that were accompanied by osteoblastic (Fig. 1*f*, arrowheads) as well as osteolytic reactions undergoing remodeling (Fig. 1*f*, arrows). Tumor cells stained positively with variable intensity by an anti-PSA antibody (Fig. 1*g*, arrow and arrowhead). We found that the tumor "take" rate was affected by neither the vehicle used for resuspension of the cells nor by the cell number (5×10^5 *vs.* 1×10^6) injected (data not shown). Although no histomorphologic evidence of tumor growth was detected in femurs of athymic mice injected with LNCaP cells, 4 of 8 animals (50%) had serum PSA levels ranging from 0.6 to 1.1 ng/ml at the end of the 4-month observation period. Similarly, one C4-2 injected athymic mouse with a serum PSA of 1.2 ng/ml had no histologically confirmed tumor growth in the bone (data not shown). C4-2 injected athymic mice had a nearly 2-fold higher tumor "take" rate and expressed much higher serum PSA levels than the C4-2B_4 injected group.

In SCID/bg mice, the intrafemoral injection of 1×10^6 cells of either LNCaP, C4-2 or C4-2B_4 cells resulted in tumor "take" rates of 6/8 (75%), 8/8 (100%) and 8/8 (100%), respectively (Table II). All animals had detectable and time-dependent elevation of serum PSA with clear histological evidence of the presence of tumor cells in the femurs (Fig. 1*h,i*, arrows). SCID/bg animals injected intrafemorally with C4-2 and C4-2B_4 cells generated tumors with areas of new osteoid deposition (Fig. 1*h,i*, arrowheads) as well as chondrocytic differentiation (Fig. 1*i*, asterisk) in contrast to old bone (Fig. 1*h,i*, arrows). The demarcation between old and new bone can be seen clearly (Fig. 1*h*, asterisks). Chondrocyte-appearing areas were not seen in LNCaP bone marrow established prostate tumors. The profiles of serum PSA elevation in athymic or SCID/bg mice injected intrafemorally with either LNCaP, C4-2 or C4-2B_4 cells are shown in Figure 2. Serum PSA was detectable significantly earlier ($p < 0.001$) in SCID/bg (3 weeks) than athymic mice (6 weeks) for all cell lines examined. These data were similar for LNCaP (Fig. 2*a*), C4-2 (Fig. 2*b*) and C4-2B_4 (Fig. 2*c*) cells with the only difference apparent in the level of serum PSA produced. Interestingly, while C4-2B_4 cells had a higher level of PSA secretion *in vitro* than C4-2 cells (data not shown), the opposite was observed *in vivo* (Fig. 2). LNCaP cells intrafemorally injected into SCID/bg required 5 weeks to have the majority of SCID/bg mice with positive serum PSA levels all of which were below those observed for C4-2 and C4-2B_4 injected animals (cf. Fig. 2*a vs.* Fig. 2*b,c*). Furthermore, LNCaP PSA values tended to decline progressively beyond the 9-week observation point. No paraplegia was observed in SCID/bg mice by 9 weeks nor in athymic mice by the end of the 4-month observation period.

All PC-3 injected mice (n = 4) developed rapidly expanding tumors without detectable serum PSA 6–10 weeks after intrafemoral injection. Marked osteolytic lesions, even total absorption of femurs, were noted using X-rays (Fig. 1*j*, arrowhead). Unlike the LNCaP sublines, the PC-3 cells formed a solid tumor mass that, as expected, showed no evidence of positive PSA immunostaining (data not shown). In contrast to the intracardially injected mice, 2 of the PC-3 intrafemorally injected mice (50%) developed ipsilateral paraaortic lymph node metastasis (data not shown).

Roentgenogram and bone scan

Roentgenographic examinations show mixed osteoblastic (Fig. 1*k*, arrowheads) and osteolytic (Fig. 1*k*, arrows) reaction in all LNCaP lineage-derived cell line injected animals. The ^{99m}Tc-MDP bone scan failed to detect bony lesions prior to the elevation of serum PSA in the animals selected for the study. Furthermore, X-ray is superior to bone scan in the detection of early skeletal lesions. Bone scan, however, did provide evidence to suggest the location of a metastatic lesion in 1/6 (18.3%) in the C4-2B_4 cells intracardially injected animals. This animal had detectable serum PSA levels 8 weeks after injection which increased steadily but slowly. ^{99m}Tc-MDP bone scan showed a hot spot on the distal femur at 11 weeks post-injection (data not shown); meanwhile, no evidence of tumor growth was identified by the roentgenogram. Intramarrow tumor growth was ascertained by histopathological examination (data not shown).

RT-PCR analysis

RT-PCR analysis of circulating PSA-secreting tumor cells was conducted for all intrafemorally injected SCID/bg mice and the results were correlated with serum PSA levels. Representative RT-PCR of circulating nucleated cells from 8 SCID/bg mouse blood samples is shown in Figure 3*a*. While all animals were serum PSA positive, only half of these were RT-PCR positive for PSA. Figure 3*b* shows that 7 of 15 animals tested (47%) were confirmed to have circulating tumor cells expressing the PSA mRNA. The C4-2 injected animals (closed squares) had a similar RT-PCR

TABLE II COMPARISON OF INTRAOSSEOUS INJECTIONS OF HUMAN PROSTATE CANCER CELLS TO EITHER ATHYMIC OR SCID/bg MICE[1]

	Athymic mice			SCID/bg
	Pericranial (5×10^5 cells)	Intrailiac (1×10^6 cells)	Intrafemoral[2]	Intrafemoral (1×10^6 cells)
LNCaP	0/3	0/4	0/8	6/8 (75%)
C4-2	0/3	4/8 (50%)	7/9 (78%)	8/8 (100%)
C4-2B_4	0/3	0/4	5/11 (45%)	8/8 (100%)
PC-3[3]		—	4/4 (100%)	—

[1]Data are expressed as positive/total number of mice (% tumor "take" rate).–[2]Tumor cells (5×10^5 or 1×10^6) were resuspended in either 50 μl of PBS or 50 μl T-medium containing 10% FBS; no apparent difference was observed in tumor "take" rate by either of these cell numbers or injecting vehicles.–[3]PC-3 cells were used as a positive control for prostate tumor growth and were shown to be osteolytic.

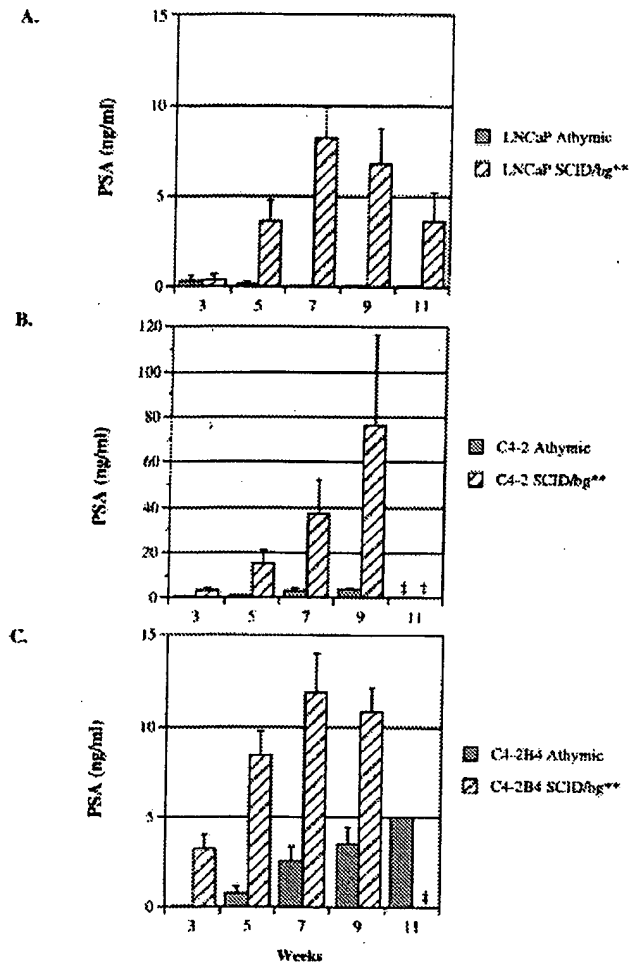

FIGURE 2 – Comparison of the serum PSA values for animals injected intrafemorally with LNCaP *(a)*, C4-2 *(b)* or C4-2B$_4$ *(c)* cell lines. Hatched bars reflect serum PSA values in SCID/bg mice. Gray bars reflect serum PSA values in athymic mice. Data are presented as values ± SEM of 8–11 animals. Both host (SCID/bg *vs.* nude) and host-dependent PSA production were measured and found to be statistically significant (**$p < 0.001$). ‡Not measured.

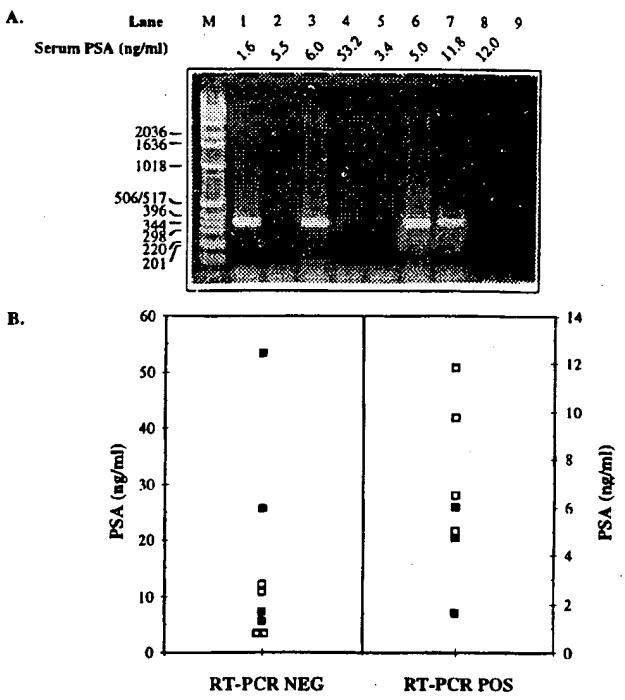

FIGURE 3 – *(a)* One percent agarose/TAE ethidium bromide-stained agarose gel electrophoresis of nested RT-PCR products using PSA-directed primers. Nucleate cell RNAs were obtained from blood samples of intrafemorally injected, serum PSA positive mice. The presence of a 355 bp product indicates the presence of circulating tumor cells. All animals were confirmed to be tumor bearing by histological examination. No correlation between circulating prostate cancer cells and serum PSA was observed. *(b)* Ranges of serum PSA values are shown. Neither the host nor the cell line used (C4-2, closed squares; C4-2B$_4$, open squares) had any effect on the lack of correlation between serum PSA positivity and the presence of circulating tumor cells.

positive rate compared to the C4-2B$_4$ injected group (open squares) (3/7, 43% *vs.* 4/8, 50%) at 5 weeks after intrafemoral injection. No correlation between serum PSA levels and the existence of circulating PSA-producing prostate cancer cells in the blood was found (Fig. 3*a,b*).

DISCUSSION

Our study describes the development of a PSA-producing skeletal growth and/or metastatic model of human prostate cancer in athymic and SCID/bg mice. The basis of this study is to introduce a series of LNCaP lineage-derived cell lines, C4-2 and C4-2B$_4$, intracardially or intraosseously to induce skeletal growth of prostate cancer cells *in vivo*. We have monitored serum PSA and circulating prostatic cells in these mice. The results show that 1) the LNCaP lineage-derived cell lines C4-2 and C4-2B$_4$, but not the parental LNCaP cells, when injected intracardially or intraosseously, consistently formed tumor growth in the skeleton; 2) C4-2 appears to be the most reliable cell line for producing intraosseous tumor growth in the femur of athymic and SCID/bg mice; 3) intraosseous growth of prostate tumors can be monitored reliably by serum PSA; histopathologic evidence of prostatic tumor growth in bones correlated perfectly with serum PSA but not with circulating prostatic cells, as detected by RT-PCR, in serum; roentgenograms and ^{99m}Tc-MDP bone scans were less sensitive than serum PSA in detecting prostate cancer growth in the skeleton; 4) LNCaP lineage-derived C4-2 and C4-2B$_4$ cells formed intrafemoral tumors more frequently and occurred with a shorter latency period in SCID/bg than in athymic mice; 5) intraosseous growth of C4-2 and C4-2B$_4$ cells in athymic and SCID mice induced marked osteoblastic responses in the skeleton. The LNCaP progression model of human prostate cancer closely mimicked the clinical manifestation and progression of human prostate cancer, and is superior to other prostate cancer models available today in evaluating bone-prostate interaction *in vivo*.

Our present study has documented that the route of tumor cell administration plays a role in determining prostate cancer cell dissemination to the bone. Thalmann *et al.* (1994) showed that C4-2 cells, when administered s.c. or orthotopically, resulted in skeletal metastasis in both intact and castrated adult male mice with a mean latency period of 6.8 months for the development of paraplegia associated with bone lesions. Neither LNCaP nor C4-2 cells, when administered i.v., gave rise to skeletal metastases during a 4-month observation period. A low incidence (20%) of skeletal metastasis was found in athymic mice when injected intracardially with C4-2 but not LNCaP cells. A higher incidence of C4-2 cell skeletal metastasis (29%) was observed when administered to SCID/bg mice (Table I). These data are consistent with the

view that skeletal metastasis of prostate cancer in experimental models occurred through the hematogenous rather than the lymphatic pathway.

Higher incidence of tumor metastasis to the skeleton by intracardial administration of tumor cells can be explained by a high tumor cell perfusion to the skeleton through this route of administration (Berrettoni and Carter, 1986). Conversely, i.v. administration often results in tumor cell arrest in the lung parenchyma, and hence decreases the probability of tumor cells reaching the bone marrow space (Fidler, 1970; Weiss *et al.*, 1988). LNCaP cells, when injected through iliac or intrafemoral routes in athymic mice, failed to form tumors within the bone marrow space, as determined histologically, but transient elevation of serum PSAs was detected in most animals. In contrast, C4-2 cells, when injected similarly, consistently formed tumors in iliac (4/8, 50%) and femur (9/16, 56%) bone marrow spaces with continuously rising serum PSA expression. When compared to C4-2 cells, C4-2B_4 formed tumors to a lesser extent in the femur (5/18, 28%) and failed to grow in the iliac bone (0/4). In SCID/bg mice, intrafemoral injection of LNCaP, C4-2 or C4-2B_4 cells formed tumors with a consistently high incidence (75%, 100% and 100% tumor "take" rates, respectively) and marked secretion of PSA into host serum. Secretion of PSA by LNCaP intrafemoral tumors tended to decline by week 9, whereas no decline was observed for either C4-2 or C4-2B_4 cells injected intrafemorally. It would appear that the anatomical site of tumor cell administration plays a key role in determining the tumor "take" rate since none of the athymic mice injected pericranially with LNCaP, C4-2 or C4-2B_4 formed tumors.

No correlation was found between circulating prostate cancer cells detected by RT-PCR analysis and the serum PSA level. Indeed, animals with positive serum PSA levels were found to be negative for circulating cells and *vice versa* (Fig. 3). These data are consistent with reports for RT-PCR from patient peripheral blood samples that also demonstrate no reliable correlation between the extent of metastatic disease and circulating tumor cells (Ghossein *et al.*, 1995; Israeli *et al.*, 1994, 1995; Moreno *et al.*, 1992). Unlike PC-3 tumors, the bone metastatic variants of the LNCaP lineage (C4-2 or C4-2B_4) induced marked osteoblastic, and to a lesser degree osteolytic, responses in the skeleton. Some evidence of increased vascularity and chondrogenesis was observed in C4-2 and C4-2B_4 injected SCID/bg hosts but not in athymic hosts. The tumor cells synthesize and secrete PSA in the bone, but the immunohistochemistry exhibits a wide variability in staining intensity. Serum PSA, however, was detected earlier in SCID/bg (3–5 weeks) than in athymic (7–10 weeks) mice. Interestingly, while PSA secretion by C4-2B_4 cells was higher than C4-2 cells in culture (data not shown), the intrafemoral osseous tumors derived from the injection of C4-2B_4 cells consistently expressed 3- to 4-fold less serum PSA than C4-2 cell-derived tumors in both athymic and SCID/bg hosts (Fig. 2). These data are consistent with the results of Thalmann *et al.* (1994), who described the lack or reduced levels of serum PSA in animals carrying C4-2B_4 and other C4-2-derived osseous metastases. These data suggest some adaptive response of the bone-derived C4-2B_4 cell line to the bone environment that results in down-regulation of PSA secretion *in vivo*. This supposition was strengthened by our observation that PSA secretion was down-regulated *in vitro* by C4-2 cells when grown in 3-dimensional coculture with bone stromal cells (data not shown). This negative interaction between prostate cancer and bone stromal cells on PSA expression is possibly coupled to the enhanced prostatic cancer cell growth as osseous metastases. This increased cell growth fits well with the hypothesis that as prostate cancer progresses, bone stromal cells may secrete factors that stimulate prostate cancer growth and down-regulate PSA expression.

There are a limited number of experimental preclinical models that have been established to study prostate cancer-bone interaction. The Mat-LyLu cell line derived from the Dunning R3327AT tumor by serial s.c. implantation in rats, when injected intracardially, induced spinal metastasis in 100% of the injected animals (Haq *et al.*, 1992). The spontaneous Pollard rat tumor (PA-3), when deposited over the calvarium or the scapular, induces osteolytic and osteoblastic changes in surrounding bone tissues (Pollard *et al.*, 1988). The PC-3 cell line, originally derived from a metastatic bony lesion, when injected orthotopically, s.c., intrasplenically or intraperitoneally (i.p.), fails to form bony metastases (Fu *et al.*, 1992; Shevrin *et al.*, 1989; Stephenson *et al.*, 1992). However, PC-3 cells, when injected via the tail vein with occlusion of the inferior vena cava, induces tumor metastasis to the skeleton (Shevrin *et al.*, 1988); SCID mice are more susceptible to skeletal metastases, which develop successful skeletal lesions in mice without inferior vena cava occlusion (Wang and Stearns, 1991). Unlike the LNCaP lineage-derived cell lines, PC-3 cells, when metastasized to the bone, induce an osteolytic reaction in the lumbar spine, pelvis, femurs, mandible and ribs. While a number of groups have developed human prostate cancer xenografts in athymic and SCID mice, as a general rule, these models seldom demonstrated the capability of the administered tumor cells to metastasize beyond localized tumor growth. However, 2 groups (Klein *et al.*, 1997; Nagabhushan *et al.*, 1996) were successful in obtaining a human prostate cancer xenograft which could progress from an androgen-dependent to an androgen-independent state, and have observed that tumor cells appear to deposit in the bone, as evidenced by RT-PCR of PSA-producing cells in the bone marrow specimens. In comparison to these previously described systems, LNCaP lineage-derived cells have the following advantages when considering the establishment of prostate cancer bone metastasis: 1) intrafemoral injection of the LNCaP lineage-derived cell line C4-2 in SCID/bg mice consistently induced osteoblastic reaction and tumor growth in the bone with detectable circulating PSA within 3 weeks; 2) LNCaP lineage-derived C4-2 and C4-2B_4 cells share a lineage relationship with the parental LNCaP cells; because of this lineage relationship, LNCaP, C4-2 and C4-2B_4 cells are excellent candidates for comparative studies of the mechanisms of prostate cancer progression from androgen dependence to androgen independence and to delineate the molecular basis of prostate cancer growth, adhesion, migration and induction of osteoblastic reaction in experimental models; 3) both the parental and the LNCaP lineage-derived C4-2 and C4-2B_4 cell sublines are capable of synthesizing and secreting PSA, and of expressing PSMA. Furthermore, these LNCaP-derived sublines, C4-2 and C4-2B_4, have undergone progressive changes from an androgen-dependent to an androgen-independent state, with increasing capability of producing PSA autonomously. The characteristics of these prostate cancer cells provides an opportunity to elucidate the role of androgen receptor in mediating ligand-dependent and -independent activation of target genes in human prostate cancer cell lines.

In summary, our results describe the development of PSA-producing, osteoblastic skeletal growth and/or metastatic models of human prostate cancer in athymic and SCID/bg mice. These models were generated by either intracardiac or intraosseous injection (through intrailiac or intrafemoral routes) with the LNCaP lineage-related sublines C4-2 or C4-2B_4. Because of the production of PSA and PSMA by LNCaP and its lineage-derived cell lines, the growth and dissemination of prostate cancer cells in these preclinical animal models can be conveniently monitored. The LNCaP progression model of human prostate cancer should be valuable for studying the molecular mechanisms of human prostate cancer progression and serve as an attractive model for the screening of potential therapeutic agents that may interfere with prostate cancer development or retard prostate cancer growth and metastasis.

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Clinical Investigation

RATE OF PSA RISE PREDICTS METASTATIC VERSUS LOCAL RECURRENCE AFTER DEFINITIVE RADIOTHERAPY

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Objective: A rising prostate specific antigen (PSA) following treatment for adenocarcinoma of the prostate indicates eventual clinical failure, but the rate of rise can be quite different from patient to patient, as can the pattern of clinical failure. We sought to determine whether the rate of PSA rise could differentiate future local versus metastatic failure.

Methods and Materials: Two thousand six hundred sixty-seven PSA values from 400 patients treated with radiotherapy for localized adenocarcinoma of the prostate were analyzed with respect to PSA patterns and clinical outcome. Patients had received no hormonal therapy or prostate surgery and had >4 PSA values post-treatment. PSA rate of rise, determined by the slope of the natural log, was classified as gradual [< 0.69 log(ng/ml)/year, or doubling time (DT) > 1 year], moderate [0.69–1.4 log(ng/ml)/year, or DT 6 months–1 year], or rapid [>1.4 log(ng/ml)/year, or DT < 6 months].

Results: Sixty-one percent of patients had non-rising PSA following treatment; 25% of patients with rising PSA developed clinical failure, and 93% of patients with clinical failure had rising PSA. The rate of rise discerned different clinical failure patterns. Local failure occurred in 23% of patients with moderate rate of rise versus 7% with gradual rise ($p = 0.0001$). Metastatic disease developed in 46% of those with rapid rise versus 8% with moderate rise ($p < 0.0001$). By multivariate analysis, in addition to rate of rise, PSA nadir and rate of decline predicted local failure; those with post-treatment nadir of 1–4 ng/ml were five times more likely to experience local failure than nadir < 1 ng/ml ($p = 0.0002$). Rapid rate of rise was the most significant independent predictor of metastatic failure.

Conclusions: The rate of PSA rise following definitive radiotherapy can predict clinical failure patterns, with a rapidly rising PSA indicating metastatic recurrence and moderately rising PSA local recurrence. This information could potentially direct therapy; if the rise predicts metastatic failure hormonal therapy could be considered, while aggressive salvage therapy may benefit subclinical local recurrence identified by a moderate rate of PSA rise.

Prostate specific antigen, Prostate cancer, Radiation therapy, PSA rise, Local recurrence, Metastasis.

INTRODUCTION

ostate cancer is the most common malignancy and sec-ıd leading cause of cancer deaths in men (4). There is wide spectrum of biological behavior, ranging from oc-ılt, low-malignant potential disease to aggressive disease ith a course that appears unaltered by therapy. This comicates the substantial debate over the most appropriate eatment modality for local therapy (10, 18, 20), and ven whether treatment is indicated (7).

PSA has emerged as a valuable tumor marker for prote cancer. It is a powerful prognosticator and helps iden-y patients who have advanced or more aggressive disıse (21, 27, 31). In addition, rising PSA following eatment has been shown to be a reliable index of eventual clinical failure (12, 15). Since comparison of treatment modalities requires prolonged follow-up prior to evidence of clinical failure, rising PSA facilitates the evaluation of local therapies by allowing comparison at an earlier endpoint (14, 16).

A rising PSA following treatment indicates eventual clinical failure, but the rate of rise varies greatly, as does the pattern of failure. When comparing treatment modalities, it is important to differentiate between local treatment failure versus clinical manifestation of pre-existing metastatic disease which is ultimately responsible for failure, regardless of the efficacy of local treatment. Subclinical indicators of failure patterns could also direct potential therapy; salvage therapy for local failure versus hormonal therapy for low volume metastatic disease.

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We recently reviewed our series of patients treated with conformal radiotherapy for localized adenocarcinoma of the prostate to determine the utility of PSA as a predictor of outcome. In confirming observations of others that rising PSA predicts eventual clinical failure, we noted a difference in the patterns of rising PSA. We investigated whether the different rates of rising PSA represented different failure patterns, and whether the rate of PSA rise could aid in predicting eventual clinical outcome.

MATERIALS AND METHODS

Patients who were treated at the University of Michigan Medical Center or Providence Hospital of Southfield, Michigan with definitive radiotherapy for localized adenocarcinoma of the prostate between 1987 and 1994 were identified from our database, and records were reviewed.

Patients were staged by complete history and physical examination, routine blood chemistries, and bone scan when indicated. All patients received pelvic CT scans as part of the treatment planning process, which were of diagnostic quality and used to evaluate pelvic adenopathy; in the rare case, elective staging pelvic lymph node dissection was performed. Patients with evidence of pelvic lymph node involvement or distant metastasis were excluded, as were patients who had received prior radical prostatectomy.

Serum PSA was measured by micro-particle enzyme immunoassay (IMX, Abbott Laboratories). Values less than 0.4 ng/ml were set at 0.4 ng/ml, to ensure reliability at the lower end of detection. Since the pattern of PSA after therapy was to be analyzed by modeling, only patients who had four or more post-treatment PSA values were analyzed, to ensure accurate fit. As the significance of pre-treatment PSA was to be evaluated, only patients who had a recorded pre-treatment PSA value within six months of treatment were assessed. In general, PSA values were obtained at routine follow-up visits and the intervals between PSA values were similar from patient to patient. To assist interpretation of PSA patterns prior to and following treatment without the confounding effects of hormonal therapy, PSA values following orchiectomy or androgen ablative hormonal therapy were excluded.

Treatment consisted of external beam radiotherapy using CT treatment planning (23, 26). Patients were treated with oblique, non-axial beams or 4-field axial beams to minimize rectal and bladder toxicity to a planning target volume generally consisting of prostate and seminal vesicles with a 1 cm margin to a dose of 55.8 Gy in 1.8 Gy fractions, followed by a boost to the prostate plus 0.5 cm margin to a total dose of 64.8–69.8 Gy. Patients with Gleason grade 7 or higher received 45 Gy in 1.8 Gy to pelvic nodal regions.

Patients were seen in follow-up every 3–6 months for physical examination (including digital rectal examination) and PSA determination. Clinical endpoints were local or distant failure as evidenced by prostate biopsy showing malignancy, a new nodule on digital rectal examination (DRE), or radiographic evidence of metastasis. Some patients received planned post-treatment prostate biopsies due to participation in a concurrent institutional study. The majority of patients with palpable abnormalities received confirmatory prostate biopsies. Metastatic work-up was performed at the time of detection of local recurrence, or as clinically indicated by follow-up examination and routine studies. Upon evidence of metastatic recurrence, patients underwent evaluation to assess for local recurrence, usually by digital rectal examination, rarely by biopsy.

PSA values measured at baseline and after radiation therapy but prior to hormonal therapy were transformed by the natural logarithm to achieve normality, which allows estimation of PSA rate using the least squares regression. The nadir was defined as the first occurrence of the lowest observed PSA post-radiotherapy. Rising PSA was defined a two or more successive values greater than the nadir, whether or not they were consecutive rises. Thus, 0.4–0.7–0.9 ng/ml is a rising PSA, as is 0.4–0.7–0.7–0.9 ng/ml. The rate of PSA decline for each patient was estimated by the slope of the least squares regression line fit to the log PSA versus time in years for PSA values from pre-treatment until nadir. Similarly, the rate of PSA rise was estimated by the slope of the regression line fit to the data collected from the post-RT nadir until most recent PSA value or hormonal therapy. If no further observation was available after the nadir, a slope of zero was assigned, corresponding to no observed PSA rise. These slopes, or rates, may also be expressed as either half-life or doubling times by dividing the ln(2) by the rate (slope) of decline or rise, respectively.

Besides these estimates of PSA rate of decline and rise, other factors considered as potential predictors of the type of recurrence were the pre-treatment PSA, nadir, and Gleason score. Adjacent logit analysis (1) was used to determine which factors were associated with each type of failure: none, local or distant. In preliminary models, each prognostic factor, except Gleason score, was included in the model as a linear and quadratic term. If the quadratic term was significant, then the factor was categorized into three levels to allow a non-linear, yet interpretable, relationship with the outcomes; otherwise, the factor was expressed as a linear predictor. The factors which did not contribute statistically to the prediction of the outcome events were removed from the model using the likelihood ratio test in a backwards variable selection procedure (3).

RESULTS

Data from 400 patients were used for this analysis, for a total of 2667 PSA values. One patient's values had no post-treatment PSA decline and were excluded. Of the remaining 399 patients, 38 patients (9.7%) had evidence of local failure, 23 (5.8%) had distant failure, and 338

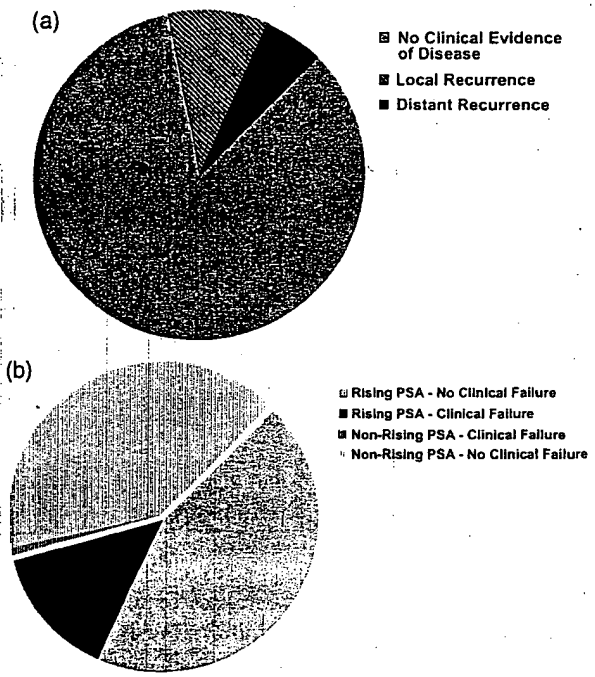

Fig. 1. (a) Distribution of clinical failure patterns. Eighty-five percent (338/399) of patients had no evidence of clinical failure, 10% (38/399) had local failure, and 6% (23/399) had metastatic failure. (b) Relationship between clinical and biochemical failure. Fifty-nine percent of patients (234/399) had rising PSA following treatment. Of those, 24% (57/234) developed clinical failure, as opposed to 2.4% (4/165) with non-rising PSA ($p < 0.0001$). Ninety-three percent of patients with clinical failure had rising PSA.

(84.7%) had no evidence of clinical failure at last follow-up (Fig. 1a). There was one patient with simultaneous local and distant failure, who was coded as distant failure. Median follow-up was 3.0 years, with a range of 0.9–7.3 years. This group of patients is representative of the 670 patients treated in our department between 1987 and 1994 with conformal radiotherapy for localized adenocarcinoma of the prostate (24). Of those not included in this analysis, 233 patients had fewer than 4 post-treatment PSA values or had received hormonal therapy, 33 patients were excluded because of insufficient clinical follow-up data, and four because there was no PSA data on record for more than one year prior to therapy.

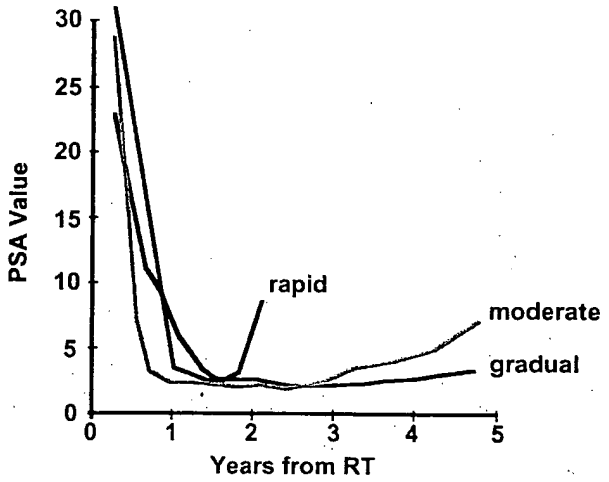

Fig. 2. Representative examples of rising PSA patterns. Rising PSA values were categorized as gradual (rate < 0.69 log (ng/ml)/year, or DT > 1 year), moderate [0.69–1.4 log(ng/ml)/year, or DT 6 months–1 year], or rapid [≥ 1.4 log (ng/ml)/year, or DT > 1 year].

Relationship between clinical and biochemical failure

Two hundred thirty-four of 399 patients (58.6%) had rising PSA values (Fig. 1b), including 57 of the 61 patients who developed clinical failure. During the time frame of this study, 57 of 234 patients (24%) with rising PSA values developed documented clinical failure. In the group of patients with non-rising PSA, only four (2.4%) developed clinical failure. This difference was significant on univariate analysis, where rising PSA predicts clinical failure ($p < 0.0001$).

PSA rate of rise

We next looked at the rate of PSA rise, and found that rising PSA values appeared to fit three patterns; those with a very gradual upward climb, those with a moderate rate of rise, and those with a rapid rate of rise. We defined a gradual rate of rise as <0.69 log(ng/ml)/year (corresponding to a PSA doubling time of >1 year), moderate rate of rise as between 0.69–1.4 log(ng/ml)/year (DT between 6 months–1 year), and rapid rate of rise as >1.4 log(ng/ml)/year (DT less than six months). Representative examples of PSA patterns are shown in Fig. 2. The distribution of rate of PSA rise and clinical failure patterns is shown in Table 1.

Table 1. Distribution of clinical failure patterns according to rate of PSA rise

	Clinical failure		
Rate of PSA rise [log(ng/ml)/year]	None (%)	Local (%)	Distant (%)
<0.69 (DT > 1 year)	288/310 (92.9)	20/310 (6.5)	2/310 (0.6)
0.69–1.4 (DT 6 months–1 year)	36/52 (69.2)	12/52 (23.1)	4/52 (7.7)
>1.4 (DT < 6 months)	14/37 (37.8)	6/37 (16.2)	17/37 (45.9)

No failure versus local failure. Some patients appear to have rising PSA for long periods of time without manifesting clinical failure. Based on the rate of PSA rise, we sought to determine whether a group of patients with a low likelihood of failure could be selected from those likely to experience local failure. Twenty of 310 patients (6.5%) with gradually rising PSA experienced local failure as opposed to 12/52 patients (23.1%) with moderately rising PSA (Fig. 3a). Comparing gradual to moderate PSA rise with regard to no failure versus local failure by adjacent category logit analysis, moderately rising PSA significantly predicts for local failure relative to no failure, with an odds ratio of 4.8 ($p = 0.0001$). In other words, patients with PSA rate of rise 0.69–1.4 log(ng/ml)/year (DT 6 months–1 year) are significantly more likely to fail locally than those with PSA rise < 0.69 log(ng/ml)/year (or DT > 1 year).

The group with PSA rate of rise < 0.69 log(ng/ml)/year contains patients with a PSA rise of zero, so it was possible that this lowest PSA "rise" had an artificially good outcome due to inclusion of patients without actual biochemical failure. We therefore re-analyzed the groups after exclusion of patients with PSA rise of 0. The difference between gradual and moderate PSA rise remained, with PSA rise 0.69–1.4 log(ng/ml)/year significantly more likely to develop local failure than PSA rise < 0.69 ($p = 0.0224$). The gradually and moderately rising PSA appear to be distinct groups with different clinical outcomes.

Distant versus local failure. We then looked at the value of rate of PSA rise in predicting distant versus local failure. Four of 52 patients (7.7%) with moderately rising PSA experienced metastatic disease as the first site of clinical failure, as opposed to 17/37 (45.9%) with rapidly rising PSA (Fig. 3b). Rate of rise > 1.4 log(ng/ml)/year was a predictor of distant as opposed to local failure when compared to rate of rise between 0.69–1.4 log(ng/ml)/year ($p < 0.001$). By adjacent category logit analysis, patients with rapid rate of rise had 8.5-fold increased odds of distant as opposed to local failure when compared to moderate rate of rise ($p = 0.0042$). Thus, rapid rate of rise predicted metastatic failure compared to moderate rise.

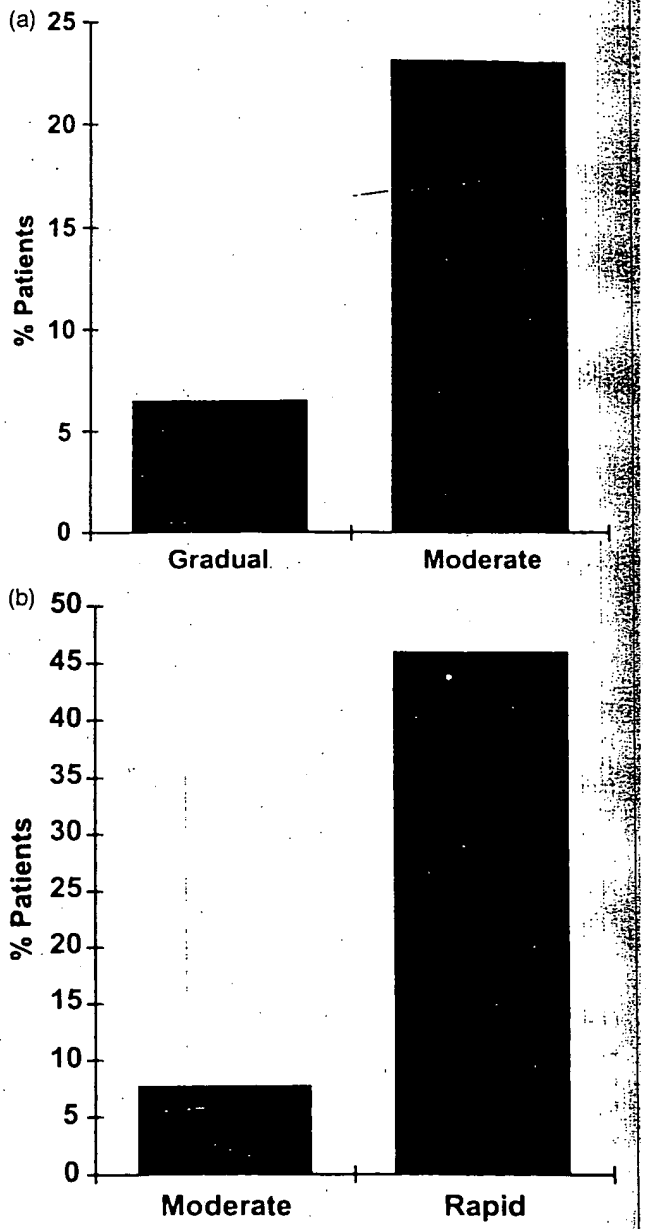

Fig. 3. PSA rate of rise correlates with clinical failure patterns. (a) Moderately rising PSA predicts local failure when compared to gradually rising PSA. Twenty of 310 patients (6.5%) with gradually rising PSA experienced local failure as opposed to 12/52 patients (23.1%) with gradually rising PSA ($p = 0.0001$). (b) Rapid rate of rise distinguishes a group with increased metastatic failure as compared to moderate rate of rise. Seventeen of 37 patients with rapid rise had metastatic failure as opposed to 4/52 with moderate rise ($p < 0.0001$).

Rate of PSA rise adjusted for additional predictive indicators

Recently, a number of potential post-treatment prognostic indicators have been identified. Since univariate analysis does not take into account the confounding effects of other risk factors, we sought to determine whether the PSA rate of rise was merely a surrogate for PSA rate of decline or nadir. Multivariate analysis was performed to adjust for nadir 1–4 versus <1 ng/ml, >4 versus 1–4 ng/ml, Gleason score < 7 versus 7 or greater, and rate of decline as a continuous variable. The rate of rise retained significance when adjusted for these other factors (Table 2); thus, the prognostic value of rate of rise is not merely a surrogate for post-therapy PSA nadir or rate of decline.

In addition, PSA nadir was found to predict local failure. Patients with a post-treatment PSA nadir of 1–4 ng/ml were significantly more likely to experience local fail-

Table 2. Results of multivariate analysis comparing failure patterns while adjusting for PSA rate of rise, nadir, Gleason score, and rate of PSA decline

	Local vs. no failure		Distant vs. local failure	
Variables	Odds ratio	*p*-value	Odds ratio	*p*-value
Moderate vs. gradual PSA rise	5.8	0.0002	5.0	0.1258
Rapid vs. moderate PSA rise	0.9	0.8583	9.6	0.0120
PSA rate of decline	0.7	0.0207	0.9	0.7542
Gleason score (≥7 vs. <7)	1.2	0.6907	4.5	0.0622
PSA nadir (1–4 vs. <1 ng/ml)	5.1	0.0002	11.0	0.0652
PSA nadir (>4 vs. 1–4 ng/ml)	2.9	0.0742	3.4	0.1253

ure than those with a PSA nadir of <1 ng/ml, with an odds ratio of 5.1 ($p = 0.0002$) (Table 2). This trend continued when comparing PSA nadir of > 4 ng/ml to 1–4 ng/ml ($p = 0.0742$). In our series, a PSA nadir of <1 ng/ml identifies a population of patients at decreased risk for local recurrence compared to those with higher nadirs. The rate of PSA decline following treatment also distinguished a group likely to experience local failure from a group unlikely to develop clinical failure ($p = 0.0207$). The faster the rate of decline, the smaller the likelihood of local failure, such that an increase of rate of decline of 1 log(ng/ml)/year decreased the odds of local failure by 0.7. While post-therapy PSA nadir and rate of decline do not diminish the ability of rate of rise to predict clinical failure patterns, they also contribute prognostic information regarding the likelihood of local failure.

We also looked to see whether pre-treatment PSA affected the ability of PSA rate of rise to predict clinical failure patterns. Although pre-treatment PSA was a significant predictor of any clinical failure, it was not significantly related to the type of clinical failure, local or distant, after accounting for rate, Gleason score, and nadir; therefore, pre-treatment PSA was not included in the final model.

With regard to distinguishing metastatic from local failure, only rapid rate of rate of rise [>1.4 log(ng/ml)/year] statistically significantly predicted metastatic disease, although nadir and Gleason score 7 or greater approached significance. Rapidly rising PSA remains the most powerful predictor of metastatic failure, even when adjusted for other prognostic indicators.

DISCUSSION

We have shown that there is a correlation between rate of PSA rise following radiotherapy and eventual clinical failure patterns. A rapid rate of PSA rise [>1.4 log(ng/ml)/year], indicates likely metastatic failure as opposed to a moderate rate of rise [0.69–1.4 log(ng/ml)/year], which is associated with local failure. Other authors have proposed PSA DT as a prognostic indicator. Hanks *et al.* found that tumor doubling times varied from 1.2 to 36 months, and that faster doubling times were associated with higher Gleason score and shorter time to clinical failure (11). The authors proposed that DT could be used to discern patients likely to receive benefit from early hormonal therapy (DT < 3.8 months) as opposed to those likely to be safely observed (DT > 18 months) (8). While our method of obtaining rate of rise of PSA is different, our results are consistent with theirs, such that a rate of rise < 0.69 log(ng/ml)/year, which corresponds to a DT of >1 year, is associated with disease which has a low likelihood of clinical failure and may be best treated with observation. Likewise, we found that the subset of patients with rate of rise > 1.4 log(ng/ml)/year, or DT < 1 year, was likely to experience metastatic failure. Partin *et al.* also demonstrated that PSA patterns following radical prostatectomy could add information in a model used to predict metastatic versus local failure (19).

Others have found correlation between Gleason score and clinical outcome (12, 29). In addition, patients with shorter DT appear to have higher median Gleason score (32, 8). In our series, Gleason score was associated with metastatic failure by multivariate analysis. We did not detect a role for Gleason score in predicting local failure as opposed to no failure. We used two categories of Gleason score in the multivariate analysis: 2–6 versus 7–10. Zagars *et al.* demonstrated a difference in local recurrence when comparing Gleason score of 2–3 versus 4–6 versus 7 versus 8–10, using four Gleason score categories as opposed to two (29). It is possible that we would have seen that Gleason score added further information if we had used four Gleason score categories.

Several previous reports have not shown an association between rate of PSA decline and clinical failure (6, 28); however, in our analysis, the rate of PSA decline added information in predicting local failure. Since we did not find that rate of decline could differentiate between distant and local failure, it could be that studies using both local and metastatic failure as a single endpoint (clinical failure) are not detecting the ability of rate of decline to predict local failure.

In addition, we found that nadir was helpful in predicting local failure, and approached significance on multivariate analysis for distant as opposed to local failure. This adds to the reports of others regarding the importance of nadir in predicting clinical failure (13, 30). We found a difference between nadir of 1–4 ng/ml versus <1 ng/ml in predicting local failure.

The importance of determining local failure is two-fold. There continues to be a debate as to the relative efficacy of surgery or radiotherapy, since there have been no well-designed randomized trials directly comparing the two modalities. Many studies have compared treatment outcome, using rising PSA as endpoint (12, 14, 15, 16, 22, 25), but patient selection remains an issue. If one modality has a higher proportion of patients with micrometastatic disease, the outcome in terms of rising PSA will be worse, yet the local therapy may have been as effective. We found that a rising PSA of 0.69–1.4 log(ng/ml)/year could predict a group who were likely to manifest local failure alone, i.e., true treatment failures. If comparisons of treatment modalities specified the proportion of patients with rising PSA, and, additionally, supplied the rate of rise, more information about local failure would be communicated than using rising PSA alone as an endpoint.

Although local salvage therapy following radiotherapy has not been overwhelmingly successful, it is possible that outcome may be improved by selecting a subset of patients with small volume local disease (17). The previous reports have shown a low likelihood of success due to high rates of metastatic failure (2, 9). In patients who have a high likelihood of local failure without micrometastatic disease, as determined by a moderately rising PSA, salvage therapy may have a better chance of success. The complication rate in the previous series have been high (5) but it is possible that in the modern setting of low-volume disease as detected by rising PSA, in the setting of patients who have been treated with conformal, small field radiotherapy the complication risk might be reduced.

Since we found that a rate of rise > 1.4 log(ng/ml)/year predicted metastatic failure, rate of rise may be useful to select patients with micrometastatic disease who would be likely to benefit from early hormonal therapy. On the other hand, patients with PSA rate of rise < 0.69 log(ng/ml)/year were unlikely to manifest clinical failure within the time frame of this study, and may be a group best served by observation, sparing the side-effects and expense of unnecessary adjuvant therapy.

In conclusion, we have demonstrated an association between the rate of rise of PSA and clinical failure patterns, where a PSA rate of rise < 0.69 log(ng/ml)/year (DT $>$ 1 year) differentiates a subset of patients likely to experience no clinical failure as opposed to local failure, and PSA > 1.4 log(ng/ml)/year (DT < 6 months) indicates likely distant failure. If confirmed by an independent dataset, this information could aid in directing treatment for patients with rising PSA following radiotherapy.

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Determinants of Prostate Cancer–Specific Survival After Radiation Therapy for Patients With Clinically Localized Prostate Cancer

By Anthony V. D'Amico, Kerri Cote, Marian Loffredo, Andrew A. Renshaw, and Delray Schultz

Purpose: Identifying pretreatment and posttreatment predictors of time to prostate cancer–specific death (PCSD) after external-beam radiation therapy (RT) was the subject of this study.

Patients and Methods: A Cox regression analysis was used to evaluate the ability of the pretreatment risk group to predict time to PCSD for 381 patients who underwent RT for clinically localized prostate cancer. Posttreatment factors analyzed for the 94 patients who experienced prostate-specific antigen (PSA) failure included the time to PSA failure, the posttreatment PSA doubling time (DT), and the timing of salvage hormonal therapy.

Results: Despite the median age of 73 years at diagnosis, 45% of patients with high-risk disease were estimated to die from prostate cancer within 10 years after RT compared with 0% ($P = .004$) and 6% ($P = .05$) for patients with low- or intermediate-risk disease, respectively. Predictors of time to PCSD after PSA failure included PSA DT ($P = .01$) and delayed use of hormonal therapy ($P \leq .002$). Nearly identical estimates of PCSD and all-cause death after PSA failure were noted for patients with a short PSA DT (ie, ≤ 12 months).

Conclusion: Prostate cancer was a major cause of death during the first decade after RT for patients with clinically localized but high-risk disease, and the cause of death for patients with a short PSA DT after RT was nearly always prostate cancer. These data provide evidence to propose the hypothesis that a short posttreatment PSA DT may serve as a possible surrogate for PCSD. Prospective validation is needed.

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PROSTATE-SPECIFIC ANTIGEN (PSA) failure after either radical prostatectomy (RP)[1] or external-beam radiation therapy (RT)[2] for patients with clinically localized prostate cancer occurs in approximately 30% to 50% of cases within 10 years after treatment and is a great source of anxiety for both patients and physicians. Therefore, in an attempt to identify patients at high risk of failure after RP or RT, investigators have developed pretreatment risk groups[3] and nomograms[4] on the basis of the relative values of time to posttreatment PSA failure after RP or RT. Yet, for whom PSA failure predicts death from prostate cancer remains unanswered. The inability to answer this question has been attributed to inadequate power in databases because of relatively short follow-up and the protracted clinical course of prostate cancer and the competing causes of mortality in this patient population.[5]

A second issue related to PSA failure after RT that has had significant ramifications on the patient's quality of life and the overall cost to the health care system is whether survival is prolonged when salvage hormonal therapy is initiated at or after PSA failure at a time when the bone scan is negative as compared with positive. Unfortunately, because of patient and physician views on this matter, a randomized trial of early versus delayed hormonal therapy after PSA failure has not been successfully completed.

Therefore, this study had two goals. The first goal was to determine whether pretreatment risk groups[3] that have been shown to stratify patients by time to posttreatment PSA failure could also stratify patients by time to posttreatment prostate cancer–specific death (PCSD). The second goal was to evaluate whether posttreatment factors could predict for time to PCSD after PSA failure. Realizing that previous investigators have shown that both the time interval to PSA failure after RP[6] and the PSA DT after RP[6] or RT[7] were predictors of time to distant failure, these factors in addition to the timing of salvage hormonal therapy were included in our analysis.

PATIENTS AND METHODS

Patient Selection

Three hundred eighty-one patients with a diagnosis of clinically localized prostate cancer and treated with external-beam RT by a single physician group at a Harvard-associated community outreach facility (St Anne's Hospital, Fall River, MA) from 1987 to 2000 constituted the study cohort. The median age of the patient population at the time of initial therapy was 73 years (range, 49 to 86 years). The pretreatment clinical characteristics of the entire study cohort and the 94 patients (25%) who sustained PSA failure are listed in Table 1.

Staging, Treatment, and Follow-Up

In all cases, staging evaluation involved a history and physical examination including a digital rectal examination (DRE), serum PSA, and a transrectal ultrasound-guided needle biopsy of the prostate with Gleason score histologic grading.[8] The prostate biopsy was performed using an 18-gauge Tru-Cut needle (Travenol Laboratories, Deerfield, IL) through a transrectal approach. All biopsy material was reviewed and assigned a primary and secondary Gleason grade by a single genitourinary pathologist (A.A.R.). Before 1996, all patients had a computed tomographic scan of the pelvis and bone scan. After 1996, patients with both a pretreatment PSA level less than 10 ng/mL and a biopsy Gleason score of ≤ 6 did not undergo radiologic staging because of the less than 1% chance that these studies would reveal metastatic disease.[9] The clinical stage was obtained from the DRE findings using the 1992 American Joint Committee on Cancer staging system.[10] Radiologic and biopsy information were not used to determine clinical stage. The PSA measurement was obtained on an ambulatory basis

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Table 1. Pretreatment Clinical Characteristics of the 381 Study Patients and 94 Patients Who Experienced PSA Failure

	All Study Patients (n = 381)		Patients With PSA Failure (n = 94)	
	Low-Risk (n = 90)		Low-Risk (n = 10)	
	No.	%	No.	%
PSA ≤ 4 ng/mL	18	20	0	0
PSA > 4-10 ng/mL	72	80	10	100
Biopsy Gleason 5-6	90	100	10	100
1992 Category T1c	50	56	6	60
1992 Category T2a	40	44	4	40
	Intermediate-Risk (n = 173)		Intermediate-Risk (n = 29)	
	No.	%	No.	%
PSA ≤4 ng/mL	11	6	1	3
PSA >4-10 ng/mL	72	42	8	28
PSA >10-20 ng/mL	90	52	20	69
Biopsy Gleason 5-6	43	25*	13	45
Biopsy Gleason 7	129	75	16	55
1992 Category T1c	80	46	12	41
1992 Category T2a	51	30	8	28
1992 Category T2b	42	24	9	31
	High-Risk (n = 118)		High-Risk (n = 55)	
	No.	%	No.	%
PSA ≤ 4 ng/mL	4	3	0	0
PSA > 4-10 ng/mL	29	25	8	15
PSA > 10-20 ng/mL	25	21	15	27
PSA > 20 ng/mL	60	51	32	58
Biopsy Gleason 5-6	36	31	14	25
Biopsy Gleason 7	40	34	19	35
Biopsy Gleason ≥ 8	42	35	22	40
1992 Category T1c	26	22	6	11
1992 Category T2a	14	12	7	13
1992 Category T2b	24	20	15	27
1992 Category T2c	54	46	27	49

NOTE. Low risk: PSA ≤10 ng/mL and biopsy Gleason score ≤6 and 1992 AJCC clinical T category T1c or T2a. Intermediate risk: PSA >10 ng/mL and ≤20 ng/mL or biopsy Gleason score 7 or 1992 AJCC clinical category T2b. High risk: PSA >20 ng/mL or a biopsy Gleason score ≥8 or 1992 AJCC clinical T category T2c.

*One intermediate-risk patient had a biopsy Gleason score of 4.

within 6 weeks of the start of RT and before radiologic studies and biopsy. All PSA measurements were made using the Hybritech (San Diego, CA), Tosoh (Foster City, CA), or Abbott (Chicago, IL) assays. PSA values before 1989 were obtained as part of an American Cancer Society–sponsored screening program.

The treatment performed was conformal RT starting in 1994. Conventional RT was performed before 1994. However, a randomized trial of conventional versus conformal RT[11] has not shown a difference in cancer control outcomes. The total median dose delivered to the prostate was 70.4 Gy (range, 69.3 to 70.4 Gy) after using a 95% normalization. A first course of RT to the prostate and seminal vesicles was prescribed for patients with either a PSA level more than 10 ng/mL or a biopsy Gleason score ≥ 7, and the median dose was 45.0 Gy (range, 45.0 to 50.4 Gy). No patient received neoadjuvant, concurrent, or adjuvant hormonal therapy.

The median follow-up for the entire study cohort of 381 patients was 4 years (range, 0.5 to 13 years) using the first day of RT as time 0. The median time interval from diagnosis to start of RT was 2 months (range, 1.5 to 3.5 months). Before PSA failure, which was defined using the American Society for Therapeutic Radiology and Oncology consensus criteria,[12] all patients generally had a serum PSA measurement and DRE performed every 3 months after RT for 2 years, then every 6 months for 3 additional years, and then annually thereafter. After PSA failure, patients were followed in rotation among radiation, medical oncology, and urology on an every-3- to 6-month basis until death. The median follow-up defining the date of PSA failure as time 0 for the 94 patients who have experienced PSA failure was 2.9 years (range, 0.5 to 9.8 years). No patient was lost to follow-up and there have been 54 deaths, 20 of which were from prostate cancer.

Determination of the Cause of Death

To be considered to have died of prostate cancer, the patient needed to have developed documented (ie, positive bone scan) metastatic disease that progressed biochemically despite having exhausted all known hormonal manipulations and to have been currently undergoing or to have previously undergone cytotoxic chemotherapy. They also had to either have clinical evidence of prostate cancer progression despite chemotherapy at the time of death or were enrolled on a hospice program for end-stage prostate cancer at the time of death. As a result, no patient was scored as dying of prostate cancer unless he had hormone-refractory metastatic prostate cancer.

Salvage Hormonal Therapy

Given the lack of information regarding whether early compared with delayed initiation of salvage hormonal therapy prolongs survival, there was no policy on when to deliver hormonal therapy after PSA failure during the study period. Therefore, this decision was left to the discretion of the treating physician. Of the 94 patients who sustained PSA failure, all had received salvage hormonal therapy at the time of this analysis. The median time from PSA failure to the start of hormonal therapy was 1.5 years (range, 0.5 to 5.1 years). A bone scan was obtained at the time of PSA failure, before and within 1 week of the initiation of hormonal therapy or at the time of clinical symptomatic progression. Of the 10 patients who received hormones at the time of a positive bone scan, one had a PSA level of ≤ 10 ng/mL or less and nine had a PSA level more than 10 ng/mL. One of these 10 men had back pain that prompted the bone scan. Hormonal therapy consisted of an orchiectomy or at least 2 weeks of a nonsteroidal antiandrogen followed by lifelong luteinizing hormone-releasing hormone agonist in two and 92 patients, respectively.

Statistical Methods

A Cox regression analysis[13] was used to evaluate the ability of previously defined pretreatment risk groups[3] to predict time to death from prostate cancer for the entire study cohort of 381 patients. Time 0 was taken as the first day of RT. A Cox regression multivariable analysis was also used to evaluate the ability of time to PSA failure, posttreatment PSA doubling time (DT), and the timing of salvage hormonal therapy to predict time to death from prostate cancer or any cause for the 94 patients who had experienced PSA failure. For this analysis, time 0 was taken as the day of PSA failure, which was defined as the midpoint between the PSA nadir and first increase.[12] For all analyses, the assumptions of the Cox model were tested and met.

The pretreatment risk group and the timing of salvage hormonal therapy were treated as categorical variables, whereas the time interval to PSA failure and the PSA DT were treated first as continuous and then as categorical variables in separate Cox regression analyses. The categories selected for the time interval to PSA failure and the PSA DT were 2 years and 12 months, respectively. These times were selected for the purpose of illustration and because, on the basis of the results of previous studies,[6,7,14] these values were suggested to be clinically useful breakpoints for predicting time to distant failure. The PSA DT was calculated assuming first-order kinetics and using a minimum of three PSA values each separated in time by a minimum of 3 months. Timing of salvage hormonal therapy was categorized as being initiated at a PSA level of ≤ 10 ng/mL and a negative bone scan versus at a PSA level of greater than 10 ng/mL and negative bone scan versus at any PSA level and positive bone scan. The PSA level of 10 ng/mL was selected as a breakpoint because all 94 patients scored as PSA failures in this study had sustained PSA failure by that PSA level.

The relative risk of PCSD and all-cause death were calculated for patients on the basis of the coefficients from the Cox regression model and reported with 95% confidence intervals (CIs). For the purpose of illustration, estimates of prostate cancer–specific survival (PCSS) and overall survival (OS) were determined using the actuarial method of Kaplan and Meier[15] and were graphically displayed. Comparisons of survivorship between groups were made using the log-rank test and an adjustment for multiple comparisons was made using the methodology of Bonferonni.[13] In order to avoid the potential for overestimating cause-specific death using the method of Kaplan and Meier[15] given the competing causes of mortality in this patient cohort,[16] the cumulative incidence method was also applied to calculate this end point in cases where PCSD and all-cause death were compared.

RESULTS

Pretreatment Prognostic Factors

The median age and follow-up for patients in the low-, intermediate-, and high-risk groups were 73, 73, and 73 years and 3.9, 3.8, and 4.2 years, respectively. The results of the Cox regression time to PSA failure analysis evaluating the pretreatment risk groups are listed in Table 2 and 10-year estimates of PCSS (100% *v* 94% *v* 55%; $P = .005$) and OS (89% *v* 79% *v* 39%; $P = .03$) are illustrated in Figs 1 and 2. The relative risk (RR) of death caused by prostate cancer was 6 (95% CI, 2.5 to 10) for high-risk compared with low- or intermediate-risk patients. There was no significant difference (all pairwise values of $P > .54$) in the estimates of non-PCSS, being 12% versus 17% versus 27% at 10 years when stratified by the low, intermediate, and high pretreatment risk groups, respectively, as noted in Fig 3.

Posttreatment Prognostic Factors

The median age and follow-up beyond PSA failure for patients in the PSA DT ≤ 12 versus more than 12 months groups were 73 and 72 years and 2.9 and 2.9 years, respectively. Similarly, the median age and follow-up beyond PSA failure for patients who received salvage hormonal therapy at the time of a negative bone scan and PSA of ≤ 10 ng/mL or greater than 10 ng/mL versus a positive bone scan were 73, 72, and 72 years and 2.9, 3.2, and 2.9 years, respectively. PSA DT as a continuous variable and the initiation of salvage hormonal therapy at the time of a positive bone scan were significant independent predictors of both time to death from prostate cancer and any cause as listed in Table 2 and illustrated in Figs 4 through 7. The lack of significance for the time interval to PSA failure on multivariable analysis can be explained by the high degree of concordance between a short time interval to PSA failure (≤ 2 years) and a short PSA DT (≤ 12 months).

Twenty patients (17 high-risk and three intermediate-risk) have died of prostate cancer and five from other causes among the 94 patients who experienced PSA failure. Of the 57 patients with a PSA DT ≤ 12 months, 18 have died, 17 (94%) from prostate cancer (15 high-risk and two intermediate-risk); whereas for the 37 patients with a PSA DT more than 12 months, seven have died, three (44%) from prostate cancer (two high-risk and one intermediate-risk). For men with a short PSA DT (ie, ≤ 12 months), estimates of PCSD and all-cause death after PSA failure were nearly identical, as shown in Figs 4 and 5,

Table 2. *P* Values from Cox Regression Analyses Evaluating Ability of Pretreatment Risk Groups to Predict Time to PCSD and Posttreatment Indicators to Predict Time to Prostate Cancer–Specific and All-Cause Death

Pretreatment Analysis	
Pretreatment Risk Group	Time to Prostate Cancer Death
Low	Baseline group
Intermediate	.03
High	.0007

Posttreatment Analysis 1*				
Posttreatment Indicators	Time to Prostate Cancer Death		Time to Death From Any Cause	
	Univariable	Multivariable	Univariable	Multivariable
Time to PSA failure	.06	.77	.25	.71
PSA DT	.0004	.01	.005	.05
Hormone 2†	.002	.04	.05	.39
Hormone 3†	< .0001	.001	.0001	.0001

Posttreatment Analysis 2‡				
Posttreatment Indicators	Time to Prostate Cancer Death		Time to Death From Any Cause	
	Univariable	Multivariable	Univariable	Multivariable
Time to PSA failure ≤ 2 years	.25	.60	.54	.67
PSA DT ≤ 12 months	.003	.03	.02	.05
Hormone 2†	.002	.02	.04	.39
Hormone 3†	< .0001	.0006	.0001	.0001

NOTE. Hormone 2: Salvage hormonal therapy initiated at a PSA level of >10 ng/mL and a negative bone scan. Hormone 3: Salvage hormonal therapy initiated at any PSA level and a positive bone scan.

*Time to PSA failure and PSA DT are continuous variables.

†Baseline group (Hormone 1) is salvage hormonal therapy initiated at a PSA level of ≤10 ng/mL and a negative bone scan.

‡Time to PSA failure and PSA DT are categorical variables; baseline groups are

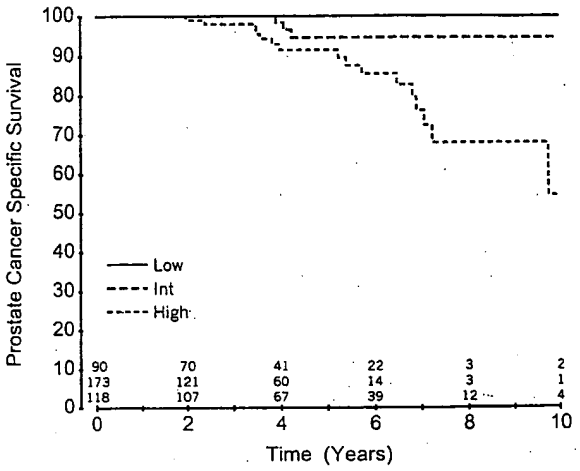

Fig 1. Prostate cancer–specific survival after RT stratified by the pretreatment risk group. Overall *P* value = .005. Pairwise *P* values: Low versus intermediate (Int), *P* = .06; Int versus High, *P* = .05; Low versus High, *P* = .004.

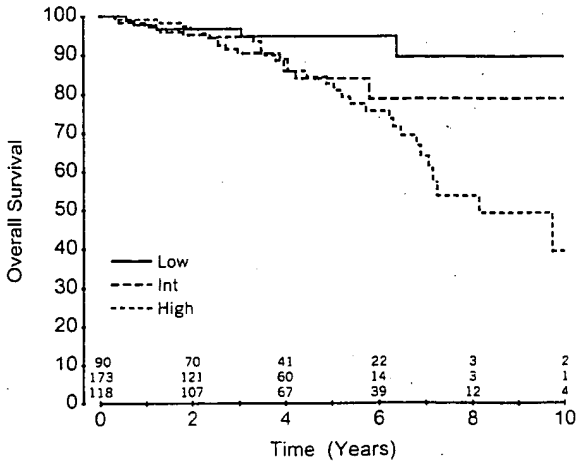

Fig 2. Overall survival after RT stratified by the pretreatment risk group. Overall *P* value = .03. Pairwise *P* values: Low versus Int, *P* = .13; Int versus High,

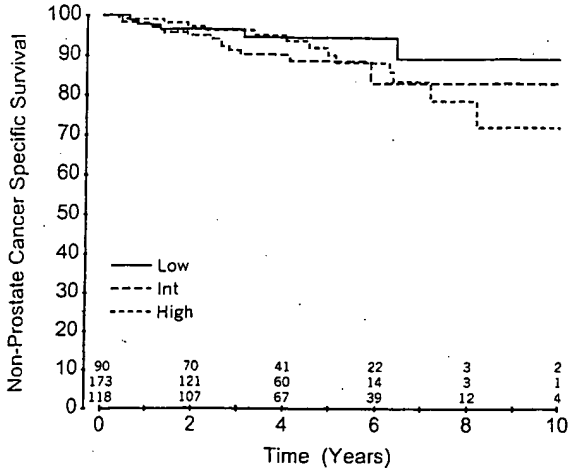

Fig 3. Non-PCSS after RT stratified by the pretreatment risk group. Overall P value = .57; all pairwise P values > .54.

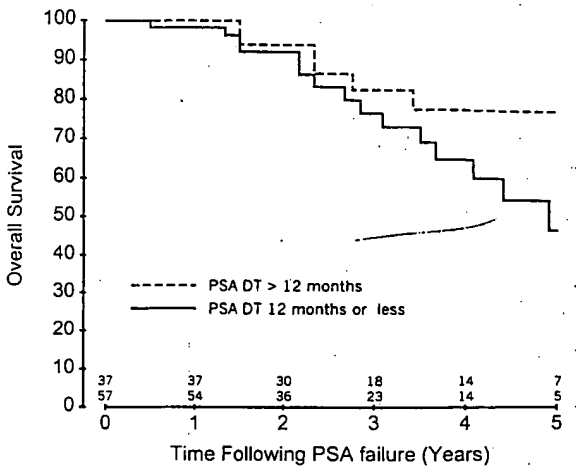

Fig 5. OS after PSA failure stratified by the PSA DT. P = .04.

respectively. This similarity in estimates of PCSD and all-cause death remained unchanged when the cumulative incidence method[16] was used to estimate PCSS in patients with a short PSA DT (ie, ≤ 12 months). Specifically, the 5-year estimate of PCSD after PSA failure was 52% and 49% using the Kaplan-Meier[15] and cumulative incidence[16] methods, respectively, both of which closely approximated the 53% 5-year estimate for all-cause death. The RR of PCSD and all-cause death was 5.1 (95% CI, 2 to 8.9; P = .03) and 2.2 (95% CI, 1.2 to 4; P = .05) for patients, respectively, with a PSA DT ≤ 12 compared with more than 12 months.

Patients who began salvage hormonal therapy when the bone scan was positive versus negative had an RR of 12 (95% CI, 6.2 to 18; P = .0006) and 9.1 (95% CI, 4 to 14.3; P = .0001) for PCSD and all-cause death, respectively. After correcting for multiple comparisons, there was a near significant increase in PCSS (P = .03) but not in OS (P = .51) if salvage hormonal therapy was initiated when the bone scan was negative with a PSA ≤ 10 ng/mL compared with more than 10 ng/mL, as noted in Figs 6 and 7. In order to minimize the potential for lead-time bias introduced by defining PSA failure as time 0, this analysis was repeated using the date of initiation of salvage hormonal therapy as time 0. The results using this approach to estimate PCSS and all-cause survival were similar to those found using PSA failure as time 0 and are illustrated in Figs 8 and 9, respectively.

To evaluate whether an imbalance in prognostic factors could have contributed to the survival differences displayed in Figs 6 through 9, the distribution of the known prognostic factors were compared for patients who initiated salvage hormonal therapy when the bone scan was positive as compared with negative. As shown in Table 3, there were no imbalances noted in the pretreatment risk group (P = .97) or the posttreatment PSA DT (P = .73) distributions among patients who received salvage hormonal therapy when the bone scan was positive versus negative.

DISCUSSION

The widespread use of monitoring serum PSA after treatment for patients with clinically localized prostate cancer has been the basis for the generation of pretreatment risk groups[3] and nomo-

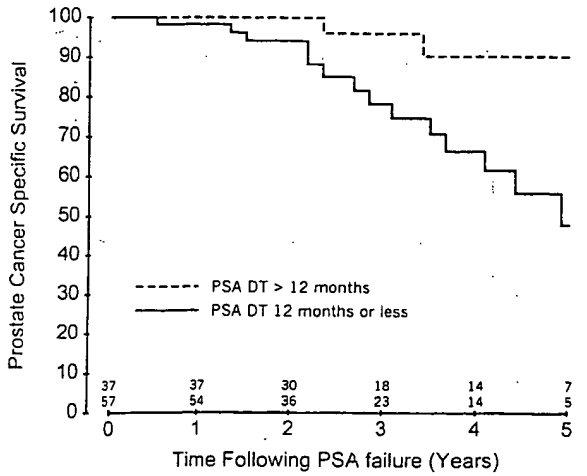

Fig 4. PCSS after PSA failure stratified by the PSA DT. P = .004.

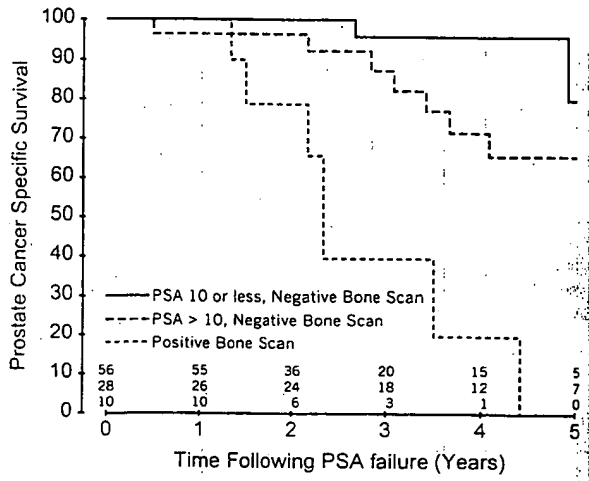

Fig 6. Cancer-specific survival after PSA failure stratified by the PSA/bone scan (BS) findings at the initiation of salvage hormonal therapy. Overall P value < .0001. Pairwise P values: PSA ≤ 10 versus > 10, BS-negative, P = .03; PSA ≤ 10, BS-negative versus BS-positive, P < .0001; PSA > 10, BS-negative versus BS-positive, P = .0006.

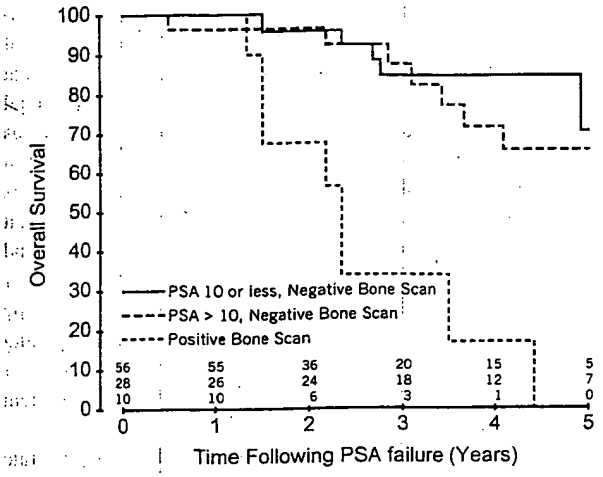

Fig 7. Overall survival after PSA failure stratified by the PSA/BS findings at the initiation of salvage hormonal therapy. Overall P value < .0001. Pairwise P values: PSA ≤ 10 versus > 10, BS-negative, P = .51; PSA ≤ 10, BS-negative versus BS-positive, P < .0001; PSA > 10, BS-negative versus BS-positive, P = .0001.

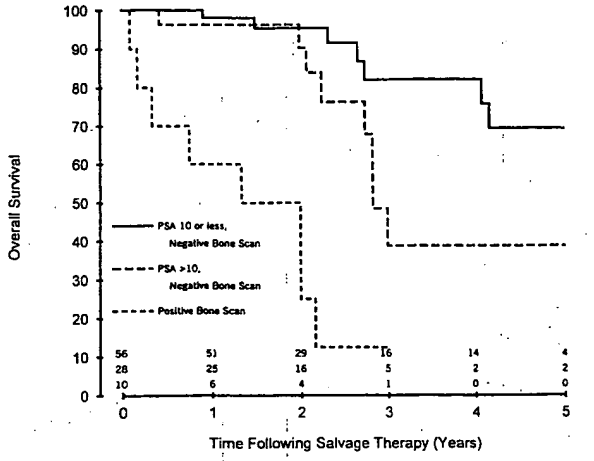

Fig 9. Overall survival after initiation of salvage hormonal therapy stratified by the PSA/BS findings. Overall P value < .0001. Pairwise P values: PSA ≤ 10 versus > 10, BS-negative, P = .02; PSA ≤ 10, BS-negative versus BS-positive, P < .0001; PSA > 10, BS-negative versus BS-positive, P = .0005.

grams[4] that provide the probability of being free from PSA failure after RP or RT. These tools have enabled the identification of men at high risk for PSA recurrence on the basis of pretreatment parameters,[3,4] posttreatment[17] parameters, or both.[18] However, it remains unclear as to whether patients who sustain PSA failure after primary therapy die of prostate cancer compared with other causes. Therefore, this study was performed to identify the determinants of PCSD on the basis of pretreatment and posttreatment predictors for men who underwent RT for clinically localized prostate cancer diagnosed during the PSA era.

The results of the study disclosed that 45% of patients with high-risk disease were estimated to have died of prostate cancer within 10 years after RT compared with 0% (P = .004) and 6% (P = .05) for patients with low- or intermediate-risk disease, respectively. Evaluating this finding in conjunction with Fig 3, where the 10-year estimate of non-PCSD was 27% for patients with high-risk disease, revealed that high-risk prostate cancer was the leading cause of mortality. This observation is particularly important, given the competing causes of mortality expected in men whose diagnosis was made at a median age of 73. Whether the addition of concurrent and adjuvant androgen suppression therapy to RT for patients with high-risk but clinically localized disease will prolong survival, as has been shown for men with locally advanced prostate cancer,[19] awaits further follow-up of completed randomized trials.

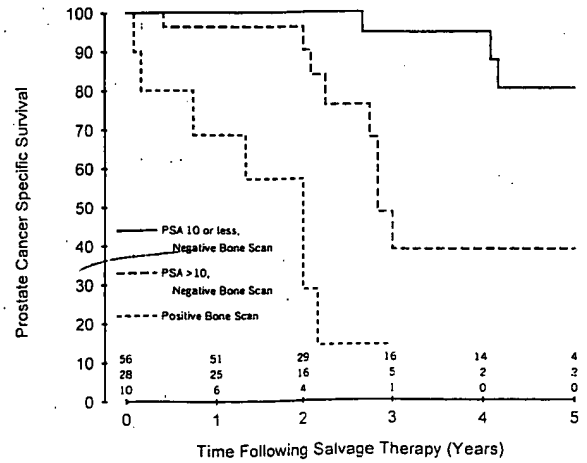

Fig 8. PCSS after initiation of salvage hormonal therapy stratified by the PSA/BS findings. Overall P value < .0001. Pairwise P values: PSA ≤ 10 versus > 10. BS-negative, P = .0005; PSA ≤ 10, BS-negative versus BS-positive, P < .0001;

The results of the multivariable analysis of posttreatment factors showed that the PSA DT when evaluated as a continuous variable was a significant predictor of both time to PCSD and all-cause death. Specifically, the study revealed that patients with a short PSA DT (≤ 12 months) had estimates of PCSD and all-cause death after PSA failure that were nearly identical, illustrating the prognostic significance of the PSA DT. Surgically managed patients are generally younger at diagnosis than the median age in this study (73 years) and also healthier. Therefore, they are less likely to have a non–prostate cancer–specific mortality profile such as that shown in Fig 3. As a result, the prognostic significance of a short PSA DT after RT may also extend to patients managed surgically, but this remains to be shown.

To further support the potential prognostic significance of the PSA DT after primary local therapy, there are now several reports from patients managed both surgically[6,20,21] and with radiation[7,14,22] suggesting that a rapid posttreatment PSA DT (6 to 12 months) is a significant predictor of time to distant failure after PSA failure. In addition, one of the radiation studies also

Table 3. Comparison of Proportion of Patients Within Each Pretreatment Risk Group and PSA DT Cohort Stratified by Bone Scan Findings at Time of Initiation of Salvage Hormonal Therapy

Risk Group	Bone Scan–Negative		Bone Scan–Positive		
	No.	%	No.	%	P
Low	9/84	11	1/10	10	.97
Intermediate	26/84	31	3/10	30	
High	49/84	58	6/10	60	
PSA DT ≤12 months	50/84	60	7/10	70	.73
PSA DT >12 months	34/84	40	3/10	30	

found the PSA DT to be predictive of time to PCSD.[14] Specifically, they estimated the 5-year PCSD to be 52% versus 10% (P = .007) for patients with a posttreatment PSA DT of approximately 1 year or less compared with greater than 1 year, respectively, similar to the results in the current study. Further follow-up of the published studies associating the posttreatment PSA DT with time to distant failure[6,7,14,20-22] will enhance our understanding of the potential prognostic significance of the posttreatment PSA DT.

To date, there has been no randomized study that has evaluated whether a difference in survival exists for the use of early as opposed to delayed salvage hormonal therapy after PSA failure after primary RT. There is evidence, however, from randomized studies that support a survival benefit to adjuvant as opposed to salvage hormonal therapy in patients with locally advanced[19] and metastatic[23] prostate cancer treated using RT and node-positive[24] prostate cancer managed with RP. Although these adjuvant therapy trials did not specifically address the question of salvage hormonal therapy, they provided the basis for the hypothesis that a prolongation in survival may be possible for early as opposed to delayed initiation of salvage hormonal therapy for patients who have experienced PSA failure after primary local therapy. Therefore, in evaluating the determinants of PCSD and all-cause death after PSA failure in this study, the question of whether the timing of salvage hormonal therapy impacted on PCSS and OS was also addressed.

The final finding in this study was a prolongation in both PCSS and OS for early (any PSA level and a negative bone scan) as opposed to delayed (any PSA level and a positive bone scan) initiation of salvage hormonal therapy for patients who had sustained PSA failure after RT. Although these data are retrospective, there were no imbalances measured in the proportion of patients within each pretreatment risk group and posttreatment PSA DT cohort for patients who began salvage hormonal therapy when the bone scan was positive versus negative, as listed in Table 3. However, a retrospective study cannot control for unknown prognostic factors, and the sample size of patients with a positive bone scan is small (n = 10). Therefore, this result awaits validation from a prospective randomized trial. Beyond the timing of the initiation of salvage hormonal therapy, other unanswered issues remain regarding salvage hormonal therapy that are not addressed in this study and include type (luteinizing hormone-releasing hormone antagonist or orchiectomy with or without a nonsteroidal antiandrogen) and duration (intermittent v continuous).

In summary, despite a median age of 73 at diagnosis, prostate cancer was a major cause of death during the first decade after RT for patients with clinically localized but high-risk disease. Moreover, the cause of death in patients with a short PSA DT (≤ 12 months) after RT was nearly always prostate cancer. Although prospective validation using Prentice's criteria[25] is needed, the data in this study provide evidence to propose the hypothesis that a short posttreatment PSA DT may serve as a possible surrogate for PCSD.

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ORIGINAL CONTRIBUTION

Natural History of Progression After PSA Elevation Following Radical Prostatectomy

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Context In men who develop an elevated serum prostate-specific antigen level (PSA) after having undergone a radical prostatectomy, the natural history of progression to distant metastases and death due to prostate cancer is unknown.

Objective To characterize the time course of disease progression in men with biochemical recurrence after radical prostatectomy.

Design A retrospective review of a large surgical series with median (SD) follow-up of 5.3 (3.7) years (range, 0.5-15 years) between April 1982 and April 1997.

Setting An urban academic tertiary referral institution.

Patients A total of 1997 men undergoing radical prostatectomy, by a single surgeon, for clinically localized prostate cancer. None received neoadjuvant therapy, and none had received adjuvant hormonal therapy prior to documented distant metastases.

Main Outcome Measures After surgery, men were followed up with PSA assays and digital rectal examinations every 3 months for the first year, semiannually for the second year, and annually thereafter. A detectable serum PSA level of at least 0.2 ng/mL was evidence of biochemical recurrence. Distant metastases were diagnosed by radionuclide bone scan, chest radiograph, or other body imaging, which was performed at the time of biochemical recurrence and annually thereafter.

Results The actuarial metastasis-free survival for all 1997 men was 82% (95% confidence interval, 76%-88%) at 15 years after surgery. Of the 1997 men, 315 (15%) developed biochemical PSA level elevation. Eleven of these underwent early hormone therapy after the recurrence and are not included in the study. Of the remaining 304 men, 103 (34%) developed metastatic disease within the study period. The median actuarial time to metastases was 8 years from the time of PSA level elevation. In survival analysis, time to biochemical progression ($P<.001$), Gleason score ($P<.001$), and PSA doubling time ($P<.001$) were predictive of the probability and time to the development of metastatic disease. An algorithm combining these parameters was constructed to stratify men into risk groups. Once men developed metastatic disease, the median actuarial time to death was 5 years. The time interval from surgery to the appearance of metastatic disease was predictive of time until death ($P<.02$).

Conclusions Several clinical parameters help predict the outcomes of men with PSA elevation after radical prostatectomy. These data may be useful in the design of clinical trials, the identification of men for enrollment into experimental protocols, and counseling men regarding the timing of administration of adjuvant therapies.

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RADICAL PROSTATECTOMY provides excellent cancer control in most men with clinically localized disease. However, approximately 35% of men will experience a detectable serum prostate-specific antigen (PSA) elevation within 10 years following surgery.[1-5] At this early sign of biochemical recurrence, patients want to know what this means, whether they will survive, and if not, how long they will have to live. Cancer-specific and metastasis-free survival rates following radical prostatectomy have been reported.[2,6-10] However, until now, the time course of progression to distant metastases or death due to prostate cancer in men with biochemical failure following radical prostatectomy has not been documented. This report characterizes the natural history of the disease in these men. This analysis provides information to men and their physicians considering systemic therapy, even in the setting of minimal elevation of PSA levels. It provides additional background data that are lacking in the proper design of some clinical trials.

METHODS

A total of 1997 men had undergone radical prostatectomy for clinically localized prostate cancer by a single surgeon at The Johns Hopkins Hospital, Baltimore, Md, between April 1982 and April 1997. The Hybritech-Tandem R and E, San Diego, Calif, and the TOSOH PSA assays, (Hybritech/Beckman, San Francisco, Calif) were used at The Johns Hopkins Hospital. These assays have been demonstrated to be comparable in

See also pp 1598 and 1642.

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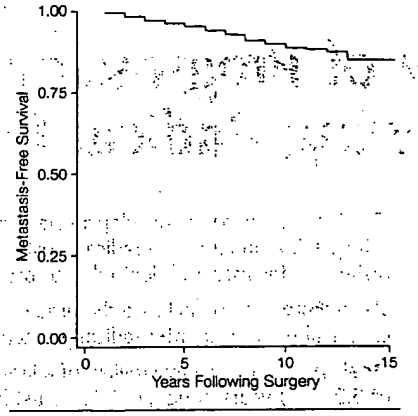
Table 1. Disease Characteristics in 1997 Men Before Undergoing Anatomical Radical Prostatectomy

Characteristic	No. (%) of Patients
TNM staging classification	
T1a	55 (2.8)
T1b	110 (5.5)
T1c	586 (29.3)
T2a	731 (36.6)
T2b	364 (18.2)
T2c	103 (5.2)
T3a	48 (2.4)
Total	1997 (100)
Serum prostate-specific antigen level, ng/mL	
0-4	487 (28.1)
4.1-10	824 (47.6)
10.1-20	326 (18.8)
≥20	96 (5.5)
Total	1733 (100)
Pathologic Gleason score	
2-4	59 (2.9)
5	380 (16.5)
6	728 (40.0)
7	655 (32.8)
8-10	155 (7.8)
Total	1997 (100)
Pathologic stage	
Organ confined	911 (45.6)
Capsular penetration with Gleason score <7	434 (21.7)
Capsular penetration with Gleason score ≥7	427 (21.4)
Involvement of seminal vesicles, negative lymph nodes	105 (5.3)
Involvement of pelvic lymph nodes	120 (6.0)
Total	1997 (100)

Figure 1. Actuarial Likelihood of Metastasis-Free Survival in 1997 Men Treated With Radical Prostatectomy



No man received early hormonal therapy prior to diagnosis of metastatic disease or symptomatic local recurrence.

intralaboratory testing. Other comparable PSA assays may have been used at referring institutions. Pathologic diagnosis of prostate cancer was based on examination of prostate tissue. Histologic grading was performed using the Gleason system for the prostatectomy specimen. No man received neoadjuvant radiation or hormonal therapy. The method of pathologic analysis at our institution has been described.[11] Tumors were determined to be organ-confined, to penetrate the prostatic capsule without extension to the seminal vesicles, to involve the seminal vesicles without nodal disease, or to involve the pelvic lymph nodes.

After the operation, men were followed up, either at our institution or by referring physicians, with serum PSA levels and digital rectal examinations performed every 3 months for the first year, semiannually for the second year, and yearly thereafter. Isolated biochemical PSA elevation was defined as a serum PSA level of at least 0.2 ng/mL, which represents a measurable value above the level of detection for this assay. Radionuclide bone scans were performed either at our institution or by the referring physicians at the time of biochemical recurrence and on a yearly basis thereafter unless performed earlier for symptoms suggestive of distant metastasis. A positive bone scan result or other radiographic or histologic (lymph node biopsy) evidence of distant failure was used for the diagnosis of distant metastases.

Thirteen men who had received immediate adjuvant radiation therapy based on pathologic features and 11 men who had received adjuvant hormonal therapy prior to the development of metastatic disease were not included in the analysis of progression after PSA elevation. Therefore, adjuvant hormonal therapy had no impact on either the time to biochemical progression or the time to distant metastasis in this analysis. Men with a PSA elevation following surgery who received postoperative radiation to the prostatic bed and demonstrated a biochemical response for longer than 24 months were considered to have local recurrences only and cured by the combination of surgery and radiation, and thus were not included in this analysis. Conversely, 83 (27%) of 304 men who had a PSA level elevation and had received adjuvant radiation without a sustained biochemical response (not cured by adjuvant radiation) were considered to harbor distant metastatic disease and were included in this analysis.

Some men with documented metastatic disease had received a variety of experimental therapies for androgen-insensitive disease. No form of systemic therapy substantially prolonged survival in men with hormone-resistant prostate cancer. These therapies were not considered to have had a significant effect on the length of survival after the development of metastatic disease.[12]

Serum PSA level increases above 0.2 ng/mL demonstrated an exponential growth curve similar to that originally reported by Patel et al.[13] By this manner, a correlation between the log of PSA levels and time was linear. Prostate-specific antigen doubling time (PSADT) was calculated by natural log of 2 (0.693) divided by the slope of the relationship between the log of PSA and time of PSA measurement for each patient. To determine the optimal PSADT cutoff for predicting metastatic disease progression for this cohort, several doubling-time calculation models were analyzed. Models that used all postoperative PSA values, only the first 2 values regardless of level,[13] only the first 2 values after a level of 0.2 ng/mL was reached, and all PSA values within a 2-, 3-, and 5-year period following a documented PSA elevation were analyzed by recursive partitioning to determine the optimal PSADT cutoff level. The method of recursive partitioning involved calculating PSADT based on the PSA values in all of the above models and using sequential values of PSADT provided by each model as a trial cutoff level to determine the optimal separation of men based on their risk of developing metastatic disease. The PSADT values that were less than 0 (stable, nonincreasing, or decreasing PSA levels) were assigned a value equal to 0. The PSADT values that were exceptionally long (eg, >100 months)

were assigned a value of 100 months for ease of calculations.

Patients who died were placed into 1 of 3 categories: dead with no evidence of disease (no previous history of a detectable PSA); dead with cancer history of a detectable PSA, and elevated PSA with documented death due to another cause), and death due to cancer. Death due to prostate cancer was defined as death in any man with metastatic disease that showed any progression following treatment with hormonal therapy. No patient with metastatic disease died due to any cause other than prostate cancer and thus cancer-specific survival was the same as overall survival in men with metastatic disease for this series.

Statistical analyses were performed using the STATA 5.0 software package (Stata Corporation, College Station, Tex). Cox proportional hazards regression analysis was used to compare the models for calculating the PSADT. The PSADT method used was optimized to provide the best χ^2 P value with the most number of men with PSA data. Analyses of actuarial survival were performed as described by Kaplan and Meier.[14] Statistical significance of Kaplan-Meier actuarial survival curves was calculated using the Wilcoxon-Gehan statistic.

RESULTS

The clinical TNM stages, the range of preoperative PSA levels, prostatectomy Gleason scores, and the pathologic stages of all 1997 men are detailed in TABLE 1. These men have been followed up for a mean (SD) of 5.3 (3.7) years (range, 0.5-15 years). Seventeen percent (344/1997) have been followed up for 10 or more years. FIGURE 1 depicts the actuarial metastasis-free likelihood following surgery for all 1997 men with a 15-year metastasis-free likelihood of 82% (95% confidence interval [CI], 76%-88%). Actuarial cancer-specific survival at 10 and 15 years following surgery was 94% (95% CI, 92%-96%) and 91% (95% CI, 87%-94%), respectively.

Three hundred fifteen men (15%) have demonstrated biochemical recurrence. No man has experienced a distant or local recurrence with an undetectable serum PSA level. Eleven of these 315 men with biochemical recurrence underwent early hormonal therapy after PSA elevation and are not included in the analysis of progression to metastatic disease. TABLE 2 depicts the pathologic stage, Gleason score, follow-up, and year of PSA recurrence for the remaining 304 men.

Various models for determining PSADT were compared using a Cox proportional hazards regression model (TABLE 3). Use of all PSA values within 2 years of initial documented PSA level elevation provided the optimal combination of statistical significance and number of evaluable men for this group. The median PSADT for this group of men (n = 131) was 10 months. When used as a cutoff level for further comparison, a PSADT of greater than or less than 10 months provided the most statistically significant prediction ($P<.001$) of time to distant disease progression after PSA elevation.

The time from PSA elevation to the development of clinically evident metastasis is depicted by actuarial analysis in FIGURE 2. The significance of Gleason score on the risk of developing metastatic disease after PSA elevation is illustrated in FIGURE 3, A ($P<.001$). At the time of this report, of the 304 men with biochemical recurrence, 103 (34%) have developed distant metastases. The median actuarial time to development of metastases following PSA elevation was 8 years, and the 5-year metastasis-free rate was 63%. Figure 3, B demonstrates that the time to development of distant metastases was dependent on the time of the PSA elevation (≤2 or >2 years following surgery; $P<.001$). Figure 3, C demonstrates that a PSADT cutoff of 10 months predicted the likelihood of subsequent development of metastatic disease as well ($P<.001$). In men with Gleason score 6 or 7 tumors, substratification according to the presence of organ-confined disease or surgical margin status did not identify a subset of men with a significantly different time course to

Table 2. Pathologic Gleason Score, Pathologic Stage, and Follow-up in the 304 Men Who Had Demonstrated Prostate-Specific Antigen (PSA) Recurrence After Anatomical Radical Prostatectomy

Variable	No. (%) of Patients
Pathologic Gleason score	
5	15 (4.9)
6	41 (13.5)
7	151 (49.7)
8-10	97 (31.9)
Total	**304 (100)**
Pathologic stage	
Organ-confined	31 (10.2)
Capsular penetration with Gleason score ≤7	30 (9.9)
Capsular penetration with Gleason score ≥7	108 (35.5)
Involvement of seminal vesicles, negative lymph nodes	52 (17.1)
Involvement of pelvic lymph nodes	83 (27.3)
Total	**304 (100)**
Years of follow-up after surgery	
0-2	304 (100)
3-5	280 (92.1)
6-9	228 (75.0)
≥10	109 (35.9)
Year of PSA recurrence	
1-2	136 (44.7)
3-5	97 (31.9)
6-9	59 (19.4)
≥10	12 (4.0)
Total	**304 (100)**

Table 3. Prostate-Specific Antigen Doubling Times and Calculation Methods*

Doubling Time Calculation	No. of Patients	Cox Regression Coefficient	95% Confidence Intervals	z	χ^2 P Value
All PSA values	228	−0.09	−0.11 to −0.05	−5.3	<.001
First 2 PSA values	212	−0.04	−0.06 to −0.02	−3.2	<.001
First 2 PSA values after 0.2 mg/mL	201	−0.03	−0.06 to −0.01	−2.6	.002
All PSA values within 2 y after recurrence	131	−0.06	−0.09 to −0.03	−3.3	<.001
All PSA values within 3 y after recurrence	91	−0.12	−0.17 to −0.07	−4.4	<.001
All PSA values within 5 y after recurrence	64	−0.04	−0.08 to −0.005	−1.7	.05

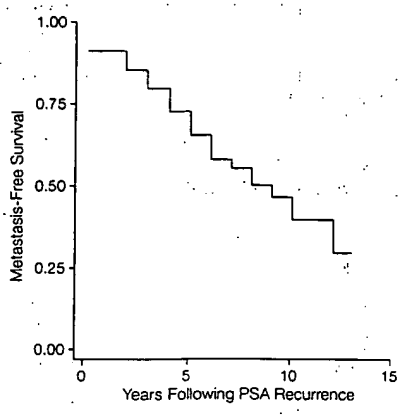
*PSA indicates prostate-specific antigen; z, the z statistic value from the Cox model; and 0.2 mg/mL, the PSA recurrence value. The Cox proportional hazards regression was used to test the predictive power of each doubling time calculation method. Time interval for the Cox regression was time from PSA recurrence to development of distant progression.

metastatic disease. When all 304 men were considered, pathologic stage stratified as organ-confined disease, capsular penetration with negative seminal vesicles and lymph nodes, or involvement of the seminal vesicles and/or pelvic lymph nodes was statistically significant in predicting time to metastatic disease (data not shown, $P = .01$).

We constructed an algorithm (FIGURE 4) to predict a man's likelihood of developing metastatic disease within various periods following initial biochemical recurrence. Unfortunately, when pathologic stage was used to further subcategorize the algorithm in Figure 4, the number of men within each category was not sufficient to obtain reasonable 95% CIs, and pathologic stage was not included in the algorithm for this reason. Using the prostatectomy Gleason score, the time of initial biochemical recurrence (≤2 vs >2 years), and PSADT (<10 vs ≥10 months) for men with Gleason score of less than 8, we estimated a man's likelihood of remaining free of clinically evident metastatic disease over various times (3, 5, and 7 years) without additional therapeutic intervention. The PSADT was not a statistically significant predictor for men with a Gleason score of greater than 7 when time to PSA elevation was known. This may be due to small numbers of men within each subset and requires further investigation. The periods indicate years from biochemical recurrence as opposed to years from surgery.

Forty-four (43%) of the 103 men with metastatic disease died due to prostate cancer. Again, no man with metastatic disease died due to any cause other than prostate cancer. The actuarial median time to death after development of metastatic disease was slightly less than 3 years (FIGURE 5). The only variable that reliably separated men based on time to death was the length of time from surgery until diagnosis of metastatic disease. FIGURE 6 demonstrates a significant difference in the time to death after development of distant disease between those men who developed metastatic disease within 1 to 3, 4 to 7, and 8 to 15 years following surgery ($P = .02$). Median survival for men developing metastases within the first 3 years after surgery was approximately 4 years from the diagnosis of metastatic disease. For men developing metastases between 4 and 7 years following surgery, median survival was approximately 5 years, and the median survival has not been reached in men developing metastases at 8 or more years following surgery. Gleason score, time to biochemical recurrence, PSADT, and serum PSA levels at diagnosis of metastases did not significantly influence time until death.

Figure 2. Actuarial Likelihood of Metastasis-Free Survival in 304 Men With Prostate-Specific Antigen (PSA) Elevation After Radical Prostectomy

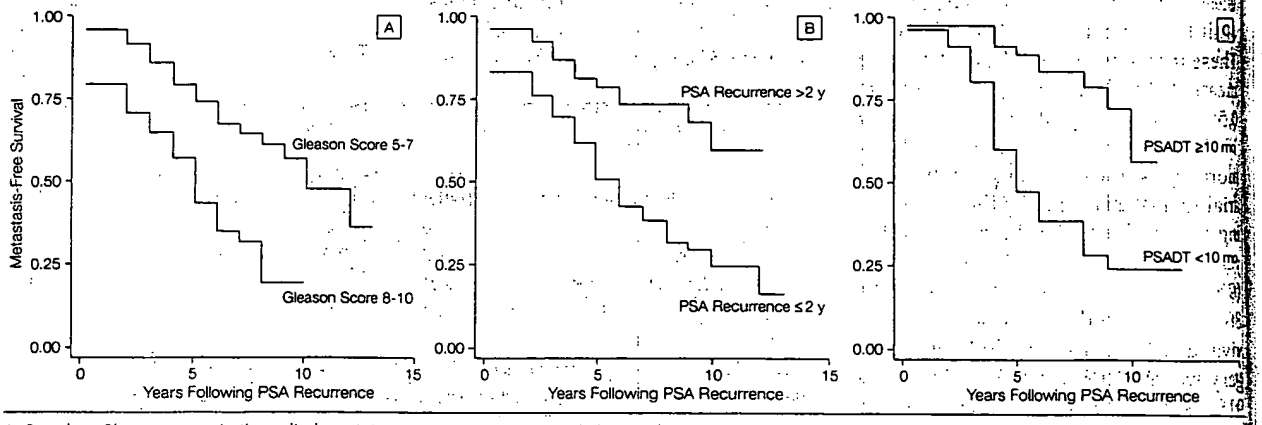


Times indicated are years from biochemical recurrence. None of the men received endocrine therapy prior to development of metastatic disease. None of the men received hormonal therapy prior to development of metastatic disease. Estimates are calculated at 3, 5, or 7 years from the time of the initial PSA elevation (metastatic disease free period), based on Gleason score in the surgical specimen, the time of initial biochemical recurrence (≤2 vs >2 years), and PSA doubling time (<10 vs ≥10 months).

COMMENT

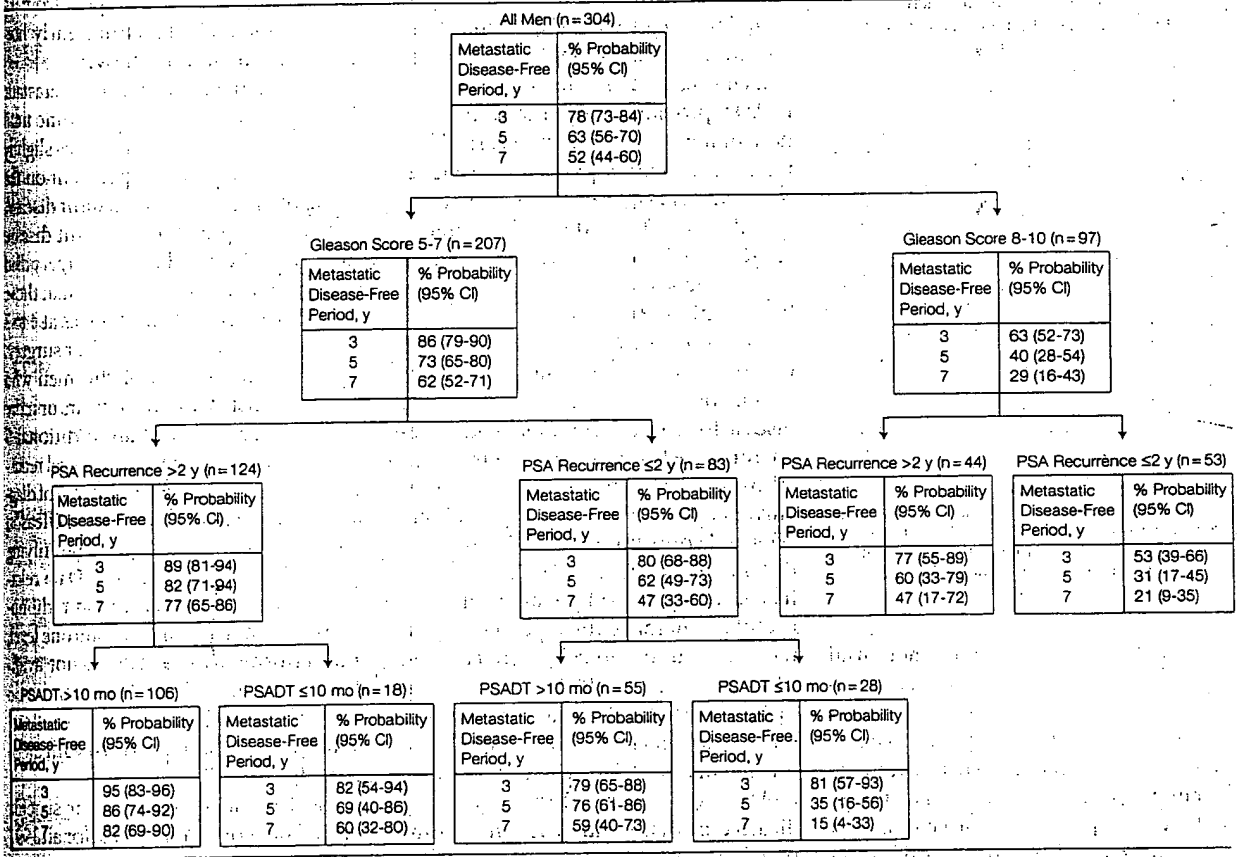
We are able to estimate a patient's probability of long-term cure after radical prostatectomy using pathologic stage as

Figure 3. Actuarial Likelihood of Metastasis-Free Survival in 304 Men With Prostate-Specific (PSA) Antigen Elevation After Radical Prostatectomy



A, Based on Gleason scores in the radical prostatectomy specimen ($P<.001$). B, Based on years until initial biochemical recurrence ($P<.001$). C, Based on prostate-specific antigen doubling time (PSADT) ($P<.001$).

Figure 4. Algorithm for Estimating a Man's Likelihood of Remaining Free of Metastatic Disease



Estimates are calculated at 3, 5, or 7 years from the time of the initial prostate-specific antigen (PSA) elevation (metastatic-disease free period, based on Gleason score in the surgical specimen, the time of initial biochemical recurrence (≤2 vs >2 years), and prostrate-specific antigen doubling time (PSADT) (<10 vs ≥10 months). CI indicates confidence interval.

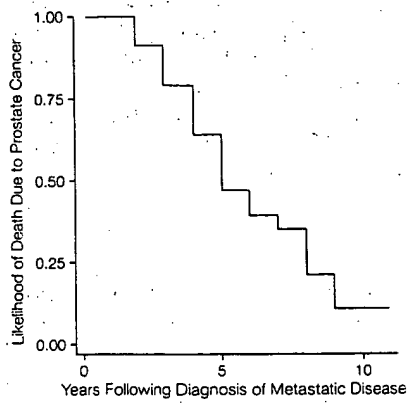
a surrogate end point. Both clinical and pathologic factors play a role in determining a patient's likelihood of having an undetectable serum PSA level at 10 to 15 years following surgery.[1-5] The most predictive of these factors include pretreatment PSA level, pathologic stage, and Gleason score; however, other microscopic features and biomarkers have also been suggested to identify patients at risk for failure following surgery.[1-5,15-17]

Between 27% and 53% of men undergoing radical prostatectomy will have a detectable serum PSA elevation within 10 years following surgery.[1-5] Until now, there have been no reliable data concerning the timing and natural history of disease progression for men with an isolated PSA level elevation after radical prostatectomy. These findings should allow physicians and patients to make educated decisions about the progression of disease and need for treatment and to facilitate the design of clinical trials.

Twenty-three percent of the men who demonstrated biochemical recurrence in our series had an undetectable serum PSA level for at least 5 years, and a small percentage (4%) had an undetectable level for 10 years prior to biochemical recurrence (Table 2). In other series, PSA progression has been rare in men with an undetectable PSA level for 5 to 6 years after surgery.[18] Our series demonstrates that with a larger number of patients and with yearly extended follow-up, men do continue to experience recurrence even 10 years and longer after surgery.

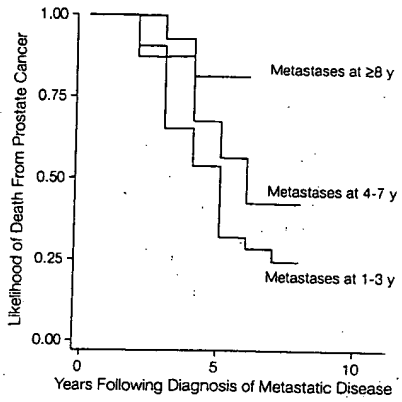
This analysis demonstrates that some men remain free of metastasis for an extended length of time after biochemi-

Figure 5. Actuarial Likelihood of Death Due to Prostate Cancer in 104 Men Diagnosed as Having Metastases After Radical Prostatectomy



The survival times indicated are years from the diagnosis of metastatic disease.

Figure 6. Actuarial Likelihood of Death Due to Prostate Cancer in 104 Men Diagnosed as Having Metastases After Radical Prostatectomy



Men are categorized according to the period after surgery in which the diagnosis of metastatic disease was made at 1 to 3 years, 4 to 7 years, or more than 8 years. The time of survival is indicated as years from diagnosis of metastatic disease.

cal recurrence. The median actuarial time from biochemical recurrence until progression to metastases was 5 years (mean, 8 years). The metastasis-free rate of 63% for all men at 5 years after biochemical progression is similar to the 60% to 75% progression-free rates at 5 years after surgery in men with lymph node–positive disease treated solely by radical prostatectomy.[19-21]

The risk of developing metastatic disease after biochemical recurrence was shown to correlate with pathologic Gleason scores. Men with tumors of Gleason scores less than 8 had a 73% chance of remaining free of progression at 5 years after biochemical recurrence compared with a 40% probability in men with higher grade tumors (Gleason score, 8-10). A similar correlation between risk of progression and tumor grade was also seen in men with lymph node–positive disease treated solely with radical prostatectomy.[20]

The length of time after surgery prior to biochemical recurrence was important in determining the risk of eventual distant failure for men with lower (5, 6, and 7) and men with high (8, 9, and 10) Gleason scores (Figure 4). A similar observation was made previously by Partin et al[22] in a report demonstrating that a high Gleason score and advanced pathologic stage were important in determining the likelihood of local or distant failure. Using a cutoff of 10 months, PSADT provided further substratification for men with a Gleason score of less than 8. Men with rapid PSA level elevation (<2 years), a Gleason score of 5 to 7, and a PSADT (>10 months) demonstrated a 76% probability of remaining free of metastatic disease for 5 years following initial PSA level elevation compared with men with a shorter PSADT (<10 months) who had only 35% chance of remaining free of metastatic disease for 5 years after biochemical recurrence. Although not as strong as Gleason score, time of biochemical recurrence, and PSADT, the pathologic stage did contribute to the likelihood of distant metastasis ($P = .01$). The PSADT has been suggested by Patel et al[13] as a useful predictor of the type of eventual recurrence after radical prostatectomy. They measured the PSADT for a group of 77 men with biochemical recurrence following radical retropubic prostatectomy and found that shorter PSADTs (<6 months) were more indicative of distant disease when compared with local recurrence.

The overall 10- and 15-year metastasis-free survival rates in the present report were 87% and 82%, respectively. Zincke et al[2] previously reported 10- and 15-year metastasis-free rates of 82% and 76% in more than 3000 men undergoing radical prostatectomy. In a multiinstitutional study, Gerber et al[6] reported 10-year metastasis-free rates that varied directly with tumor grade (low, 87%; intermediate, 68%; and high, 52%). When patients in our series were divided into these same categories, the metastasis-free rates at 10 years were better than those reported for those who had the low-grade tumors (100%) and intermediate-grade tumors (91%) but were somewhat lower in the higher-grade tumors (43%). This lower rate for the high-grade tumors may be due to the lack of early hormonal therapy in our patients with high-grade disease. Although this issue was not specifically addressed by Gerber et al,[6] more than one quarter of the men in the report from the Mayo Clinic received either early hormonal or radiation therapy.[2]

After the development of metastatic disease, the actuarial median time until death due to prostate cancer was slightly less than 5 years and dependent on the timing of progression to distant disease. Men who progressed to distant disease within 1 to 3 years following surgery died due to cancer at a higher rate than those men who developed metastases at 4 to 7 years or more than 8 years after surgery. Seventy-eight percent of the men who developed distant disease 8 years or more after surgery survived an additional 5 years. Time to original biochemical recurrence, serum PSA level at the time of diagnosis of metastatic disease, and Gleason score did not prove useful in stratifying risk of cancer-specific death. Data relating to the extent of disease on radionuclide bone scan, serum testosterone level, and performance status was not available in these men. These factors have also been shown to be important in determining overall survival in men with metastatic disease.[23,24]

The 10- and 15-year cancer-specific survival rates of 94% and 91% for all 199 men were similar to those reported in recent analyses.[7-10] As was the case for metastasis-free survival, our rates of cancer-specific survival are higher than those reported by Gerber et al[6] and Zincke et al[2]; this may due to patient selection.

None of the men in our study with metastatic disease died due to causes other than prostate cancer. This means that our cancer-specific death rate in men with metastatic disease is the same as the overall survival rate. The overall survival rate of 43% at 5 years was almost identical to that reported in men with minimal metastatic disease and good performance status in the National Cancer Institute Intergroup Study Number 0036 and other studies.[24-33]

For men who experience an isolated biochemical recurrence, the algorithm in Figure 4 should provide a reasonable estimate of their probability of developing metastatic disease over the next 3, 5, or 7 years. This information should

llow physicians and patients to make ducated treatment decisions based on heir risk of recurrence.[25-32]

We anticipate that this algorithm hould provide valuable information for he stratification of patients into different risk groups when designing and nrolling patients in investigational protocols. This analysis demonstrates that he duration of survival in these men s quite long and must be taken into account when determining the feasibility of proposed clinical trials.

ONCLUSION

This report characterizes the natural istory of disease progression to distant metastasis and death due to prostate cancer in men with a PSA elevation following radical prostatectomy. Radical prostatectomy was shown to provide excellent long-term cure rates with 82% metastasis-free survival at 15 years following surgery for all men in this study group. Of the men who did develop a PSA elevation, many remained free of metastatic disease for an extended period after initial biochemical recurrence without other forms of therapy. This has important implications in the selection of systemic therapies that are not curative and have no demonstrated impact on eventual outcome. The extended interval between biochemical recurrence and clinical metastatic disease emphasizes the need to design clinical trials to examine new treatment modalities in these men.

Factors that predicted the time course to the development of metastatic disease included the timing of initial PSA elevation, Gleason score, and PSADT. These factors were used to construct an algorithm that should be useful to the clinician in counseling patients about the time course and likelihood of eventual development of metastatic disease after initial biochemical recurrence.

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Original Article

PSA Doubling Time as a Predictor of Clinical Progression After Biochemical Failure Following Radical Prostatectomy for Prostate Cancer

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• *Objectives*: To characterize the clinical progression of disease in men who have undergone prostatectomy for clinically localized prostate cancer and have postoperative biochemical failure (elevated prostate-specific antigen [PSA] level) and to identify predictors of clinical disease progression, including the possible effect of PSA doubling time (PSADT).

• *Patients and Methods*: Between 1987 and 1993, 2809 patients underwent radical retropubic prostatectomy for clinically localized (≤T2) disease. In our database, all patients with postoperative biochemical failure (PSA level ≥0.4 ng/mL) were identified. The PSADT was estimated using log linear regression on all PSA values (excluding those values determined after administration of hormonal therapy) within 15 months after biochemical failure. All patients had regular PSA measurements from the time of surgery through the follow-up period. Systemic progression (SP) was defined as evidence of metastatic disease on a bone scan. Local recurrence (LR) was defined on the basis of digital rectal examination, transrectal ultrasonography, and biopsy. The SP-free survival and LR/SP-free survival (survival free of both LR and SP) after biochemical failure was estimated with use of the Kaplan-Meier method. Patients with prostate cancer treatment after biochemical failure had their follow-up censored from this study at the time of treatment.

• *Results*: Postoperative biochemical failure occurred in 879 men (31%). The mean follow-up from time of biochemical failure was 4.7 years (range, 0.5-11 years). The mean time to biochemical failure was 2.9 years (median, 2.4 years). The overall mean SP-free survival from time of biochemical failure was 94% and 91% at 5 and 10 years, respectively. The mean LR/SP-free survival was 64% and 53% at 5 and 10 years, respectively. By using univariate analysis on the 587 patients with PSADT data, significant risk factors for SP were PSADT ($P<.001$) and pathologic Gleason score ($P=.005$); for LR/SP, significant risk factors included PSADT ($P<.001$) and pathologic Gleason score ($P<.001$). In multivariate Cox models analysis, only PSADT remained a significant risk factor for both SP and LR/SP ($P<.001$). Mean 5-year SP-free survival was 99%, 95%, 93%, and 64% for patients with PSADT of 10 years or longer, 1.0 to 9.9 years, 0.5 to 0.9 year, and less than 0.5 year, respectively; the respective mean LR/SP-free survivals were 87%, 62%, 46%, and 38%. The percentage of patients with PSADT of less than 0.5 year was considerably higher if the type of first clinical event was SP (48%) compared with LR (18%) ($P<.001$).

• *Conclusions*: For patients who have undergone radical prostatectomy, a rising PSA level suggests evidence of residual or recurrent prostate cancer. Many men remain free of clinical disease for an extended time after biochemical failure following radical prostatectomy for clinically localized prostate cancer. The PSADT appears to be an important predictor of SP and also of any clinical progression (local or systemic). These data may be useful when counseling men regarding the timing of adjuvant therapies.

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LR = local recurrence; PSA = prostate-specific antigen; PSADT = prostate-specific antigen doubling time; RRP = radical retropubic prostatectomy; SP = systemic progression

Approximately one third of prostate cancer patients with clinically localized disease treated with radical prostatectomy develop a biochemical failure (ie, postoperative detection of serum prostate-specific antigen [PSA] level ≥0.4 ng/mL)[1] during long-term follow-up.[2-6] Moreover, PSA progression does not seem to be limited by time.[7] Although biochemical failure represents the earliest evidence of persistent disease, it does not necessarily translate into prostate cancer mortality. The course of disease in patients with biochemical failure following radical prostatectomy has been poorly understood in part due to the long-term follow-up necessary to characterize fully the often protracted course of prostate cancer.[7] However, recent reports have identified 3 important variables predictive of how long a patient may remain free of metastasis after biochemically detected recurrence: Gleason score, time interval to PSA elevation, and PSA doubling time (PSADT).[8-10] We report long-term follow-up on a large number of patients at a single institution who had biochemical failure following radical retropubic prostatectomy (RRP) for

For editorial comment, see page 571.

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clinically localized (≤T2) prostate cancer during the PSA era (1987-1993). Our goals were to describe the course of prostate disease after biochemical failure, specifically looking at the time between biochemical failure and presumed systemic progression (SP) and local recurrence (LR).

PATIENTS AND METHODS

A total of 2809 patients were treated with primary bilateral pelvic lymphadenectomy and RRP at Mayo Clinic, Rochester, Minn, for clinically localized (cT1/2) adenocarcinoma of the prostate between 1987 and 1993. All patients were entered into the Mayo Clinic radical prostatectomy database. Pathologic staging and grading were performed on histopathologic examination of the entire specimen, as previously described.[11] Histopathologic grading was performed according to the Gleason grading system.[12] The 1997 TNM staging system was used for clinical and pathologic staging.[13] No patient received neoadjuvant hormonal or radiation therapy. Patients were followed up with serum PSA measurements on a regular basis from the time of surgery throughout the follow-up period. Biochemical failure was defined as a serum PSA level of 0.4 ng/mL or higher. Clinical SP was defined as demonstration of metastatic disease on a bone scan. Local recurrence was defined as prostate cancer identified by digital rectal examination, transrectal ultrasonography, or biopsy at or in the immediate vicinity of the vesicourethral anastomosis.

The first PSA value (more than 30 days after RRP) of 0.4 ng/mL or higher was defined as the "event PSA." The PSADT was calculated with use of the slope from the within-patient linear regression of log-e of PSA (y) ys time of PSA measurement (x). The slope was estimated in 2 ways for each man: (1) by using the event PSA and all subsequent PSAs from 30 days to 15 months after the event PSA, and (2) by using the event PSA and the next PSA. The PSA determinations preceded by adjuvant therapy were excluded. The doubling time was estimated as 0.693 divided by the slope. For patients with stable PSA levels, the doubling time was arbitrarily set at longer than 10 years. The PSADTs could be calculated only for patients with at least 2 valid PSA measurements. These patients comprise the "doubling time cohort."

The PSA event cohort comprised 879 of the 2809 men in the prostatectomy cohort who had a follow-up PSA event. Survival free of SP and survival free of both LR and SP (LR/SP) after a PSA event were estimated with use of the Kaplan-Meier method; patients without progression at time of last contact or death were censored from the analysis. Unless otherwise noted, follow-up time was censored at the time of additional prostate cancer therapy. After the PSA event, 47% of patients had additional therapy in the absence of SP and 30% in the absence of both LR and SP.

The doubling time cohort (n=587) comprised patients from the PSA event cohort in whom sufficient data were available for doubling time calculations. The PSA event cohort and the doubling time cohort were nearly identical with respect to tumor pathologic stage, Gleason score, tumor DNA ploidy, and preoperative PSA (Table 1). Within the doubling time cohort, the Cox proportional hazards model was used to model SP-free and LR/SP-free survival after a PSA event as a function of pathologic Gleason score, stage, DNA ploidy, pre-RRP PSA level, interval from RRP to PSA event, and PSADT. The rank sum test was used to compare doubling times by type of first clinical event (LR vs SP). All tests were 2-sided with α=.05.

RESULTS

Table 1 shows the pathologic stages, Gleason scores, DNA ploidy status, and preoperative PSA in all patients in each cohort. A total of 879 men (31% of the RRP cohort) had biochemical failure during the follow-up period. The mean time from RRP to biochemical failure was 2.9 years (median, 2.4 years). Of those who developed biochemical failure, 84% did so within 5 years of their surgery. In the cohort of 2809 men, the median number of PSA measurements during the follow-up time was 1.03 per man per year. In the PSA event cohort, follow-up after the initial PSA event was at a median rate of 1.59 per man per year. The mean follow-up from the time of biochemical failure was 4.7 years, with a range of 0.5 to 11 years. Four patients had concurrent diagnosis of SP and PSA event, while 1 patient had no additional follow-up after the PSA event. Among the remaining 874 patients, 81 developed SP on follow-up with SP occurring in 41 patients without prior adjuvant therapy. The mean ± SE SP-free survival at 5 and 10 years from the time of biochemical failure was 94%±1% and 91%±4%, respectively, when follow-up at the time of additional prostate cancer therapy was censored (41 SP events) compared with 90%±1% and 83%±2% when follow-up treatment was ignored (81 SP events). For LR/SP-free survival, the values were 64%±3% and 53%±4%, respectively (Figure 1).

Two techniques were compared to determine PSADT: one using log linear regression for only the first 2 PSA values and the other using log linear regression for all PSA values within 15 months of the initial PSA determination of 0.4 ng/mL or higher. Using the first 2 values, we found 19%, 17%, 19%, and 45% of men to have PSADT of 0.5 year, 0.5 to 1.0 year, 1.0 to 9.9 years, and 10 years or longer, respectively. With inclusion of all PSA values, the distribution was 15%, 23%, 26%, and 36%. Agreement based on these categories was 79%, while the rank correlation coefficient for the exact times was 0.85.

Figure 2 depicts SP-free survival from the date of biochemical failure stratified by PSADT. With inclusion of all

Table 1. Cohort Comparison*

	RRP cohort (N=2809)	PSA event cohort (n=879)	Doubling time cohort (n=587)
Tumor stage			
T2a	28	20	19
T2b	40	31	32
T3a	20	24	24
T3b	11	23	22
TX N+	1	3	3
Gleason score			
2-5	53	34	34
6	20	24	24
7	23	33	34
8-10	4	10	8
Unknown (No.)†	(131)	(51)	(31)
DNA ploidy			
Diploid	78	68	68
Tetraploid	18	25	24
Aneuploid	5	7	8
Unknown (No.)†	(211)	(59)	(40)
Preoperative PSA (ng/mL)			
<4	21	11	11
4.0-9.9	47	39	39
10.0-19.9	22	30	29
≥20	11	20	21
Length of follow-up, median (25th-75th percentile) (y)			
From RRP	7.0 (5.8-8.5)	7.5 (6.2-9.4)	7.6 (6.2-9.7)
From event PSA	NA	4.6 (2.5-6.8)	5.04 (2.9-7.3)

*Values reported are percentages unless indicated otherwise. Totals may exceed 100% due to rounding. NA = not available; PSA = prostate-specific antigen; RRP = radical retropubic prostatectomy.
†Patients with unknown status not included in calculation of percentages.

PSA values within 15 months of the biochemical failure, the mean ± SE 5-year SP-free survival for patients with a PSADT of less than 0.5 year, 0.5 to 1.0 year, 1.0 to 9.9 years, and 10 years or longer was 64%±12%, 93%±3%, 95%±3%, and 99%±1%, respectively (P<.001). Among the other variables, including time interval to PSA progression, DNA ploidy, preoperative PSA level, pathologic stage, and pathologic grade, only Gleason score (P=.005) was univariately associated with SP-free survival (Table 2). By using multivariate analysis (including PSADT, Gleason score, and DNA ploidy), only the effect of PSADT was significant (P<.001). The risk ratio for Gleason score dropped from 1.7 (P=.005) to 1.3 (P=.11) when adjusted for PSADT and DNA ploidy.

Univariate analysis found only PSADT (P<.001) and Gleason score (P<.001) to be significantly associated with LR/SP-free survival; DNA ploidy (P=.15) was not significant. The mean ± SE 5-year LR/SP-free survival for patients with PSADT of less than 0.5 year, 0.5 to 1.0 year, 1.0 to 9.9 years, and 10 years or longer was 38%±11%, 46%±6%, 62%±6%, and 87%±3%, respectively (P<.001; Figure 3). By using multivariate analysis (including PSADT, Gleason score, and DNA ploidy), only the effect of PSADT was statistically significant (P<.001), although Gleason score was not (P=.054). The risk ratio for Gleason score dropped from 1.29 to 1.15 when adjusted for PSADT and DNA ploidy.

The type of first event was LR for 134 patients, SP for 23, and both (within 30 days) for 1. The percentage of patients with PSADT of less than 0.5 year was 18% if the first event was LR compared with 48% if it was an SP (P<.001). Of those patients with a PSADT of 10 years or longer, only 17 patients had a steadily rising PSA level, whereas the remainder had stable or fluctuating PSA values during the follow-up period.

DISCUSSION

The majority of men undergoing radical prostatectomy for clinically localized (T1/2) prostate cancer are cured by the procedure; however, in approximately one third of men, a PSA elevation develops during long-term follow-up.[2-7] Data are lacking regarding how best to manage these men with a

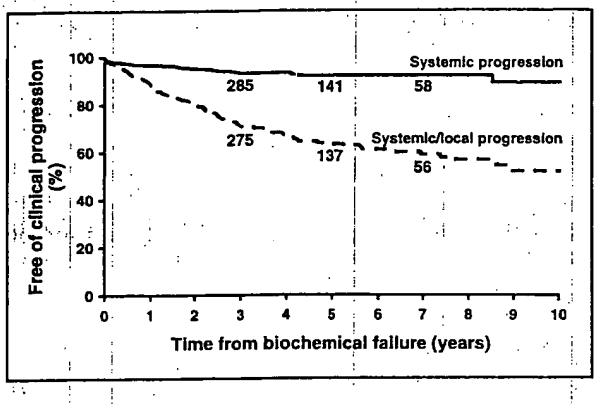

Figure 1. Systemic progression–free survival from time of biochemical failure with follow-up censored at time of adjuvant therapy.

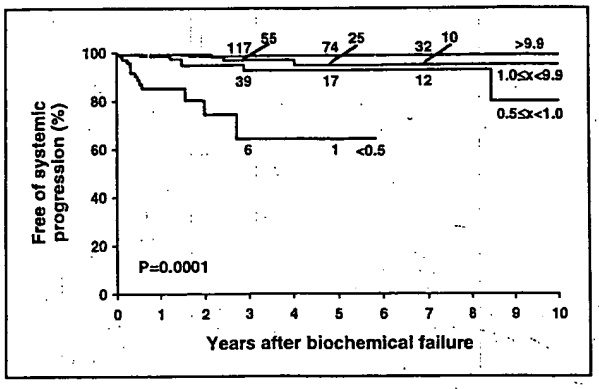

Figure 2. Systemic progression–free survival from time of biochemical failure stratified by prostate-specific antigen doubling time with follow-up censored at time of adjuvant therapy.

PSA relapse; no prospective trials have been performed, and until recently, the long-term risk of clinical progression in these patients was unknown.[8,9] In this study it was our intent to characterize the course of prostate cancer after the development of a biochemical failure (PSA level ≥0.4 ng/mL) following RRP with respect to clinical progression and to identify risk factors for clinical progression.

To our knowledge this series comprises the largest group of men (2809 patients) from a single institution who underwent radical prostatectomy for clinically confined (cT1/2) prostate cancer during the PSA era. Eight hundred seventy-nine (31%) of the 2809 men developed a biochemical failure with long-term follow-up. The interval from RRP to biochemical failure was variable in our series, with 49% of men developing a biochemical failure more than 2 years after surgery and 16% presenting more than 5 years after surgery. These time spans are consistent with the variable intervals reported in other series.[5,7-9] Clearly, a biochemical failure postoperatively does not translate into universal rapid patient demise. It has been reported that early biochemical failures (ie, within 1 year after surgical treatment) are much more likely to represent distant metastases, whereas a biochemical failure occurring more than 3 years after radical prostatectomy is much more likely to represent a local recurrence.[3,7,9,14] Irrespective of when the PSA becomes detectable, our results indicate that actual follow-up PSA data allowing calculation of the PSADT provide an excellent way to predict the likelihood

Table 2. Assessment of Risk Factors for the Development of Systemic Progression After Biochemical Failure*

Risk factor	Univariate RR (95% CI)†	Univariate *P* value	Multivariate RR (95% CI)†	Multivariate *P* value
PSADT (y)				
<0.5 (vs ≥10)	102 (12.8-819)	<.001	62 (7.6-498)	<.001
0.5-0.9 (vs ≥10)	13 (1.5-107)	.02	9.1 (1.1-79)	.045
1.0-9.9 (vs ≥10)	5.2 (0.5-51)	.15	4.1 (0.4-39)	.23
Gleason score (unit increase)	1.7 (1.2-2.4)	.005	1.3 (0.9-1.9)	.11
Nondiploid DNA ploidy (vs diploid)	1.9 (0.8-4.3)	.13	1.3 (0.5-3.2)	.56
Tumor stage				
ECE (vs OC)	1.4 (0.5-3.6)	.50	NI	...
SV or nodes (vs OC)	1.2 (0.4-3.4)	.75	NI	...
Preoperative PSA (2-fold increase)	1.0 (0.7-1.3)	.89	NI	...
Time interval RRP to biochemical recurrence (1-y increase)	0.9 (0.7-1.1)	.32	NI	...

*CI = confidence interval; ECE = extracapsular extension only; NI = not included in the model; OC = organ confined; PSA = prostate-specific antigen; PSADT = prostate-specific antigen doubling time; RR = risk ratio; RRP = radical retropubic prostatectomy; SV = seminal vesicles involved.

†Risk ratios for systemic progression for those with, relative to those without, the risk factor as estimated using univariate and multivariate Cox proportional hazards model.

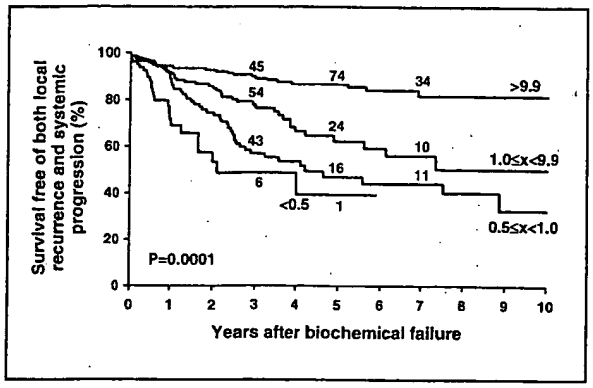

Figure 3. Survival free of both local recurrence and systemic progression from time of biochemical failure stratified by prostate-specific antigen doubling time.

of early clinical progression after a biochemical failure. Those at risk of early development of metastatic disease make up the group that would be most important to identify for early adjuvant therapy. In a univariate Cox model analysis that included time to PSA elevation, pathologic Gleason score, DNA ploidy status, preoperative PSA level, and pathologic stage as variables, the PSADT was the strongest predictor of time to metastatic disease. Patients with a PSADT of less than 0.5 year had a 5-year freedom from SP of only 64%. In contrast, the 5-year freedom from SP with a PSADT of longer than 1 year was 95%. Our results confirm those of Patel et al[9] who found that PSADT was a better indicator of time to clinical progression than was time to biochemical failure, preoperative PSA level, pathologic grade, or pathologic stage. Pruthi et al[10] concluded that PSADT and time from surgery to biochemical failure are indicative of different biological characteristics of recurrent prostate cancer. That is, PSADT appears to represent the aggressiveness of the original tumor, whereas time to biochemical failure reflects the extent of residual disease following radical prostatectomy. Pound et al[8] combined PSADT, Gleason score, and time to biochemical recurrence in an algorithm to predict a man's likelihood of developing metastatic disease. This analysis revealed an 8-year mean actuarial time from biochemical recurrence to metastasis and a 5-year mean actual time from metastasis until death.

The PSADT is calculated by taking the natural log of 2 (0.693) and dividing it by the slope of the natural log PSA line, where the natural log PSA line is derived from the linear regression of natural log of PSA (y-axis) on the time of PSA measurements (x-axis). More simply, the PSADT can be thought of as the time that it takes for the serum PSA level to double. At the very least, 2 PSA determinations separated by time are required to establish the PSADT. We explored 2 methods to calculate PSADT: (1) by using the first detectable PSA value higher than 0.4 ng/mL and the second PSA value only, and (2) by using a log linear regression of all PSA values within a 15-month period following the first detectable PSA. Two percent of patients designated as having a PSADT of less than 1 year calculated by using all PSA values in a log linear regression were assigned a PSADT of longer than 1 year when only the first 2 values were used in the calculation. One would expect more accuracy when all PSA values were used; however, it would be much simpler to use only 2 values. The calculation of PSADT for 2 values is demonstrated in the following example:

On follow-up after RRP, this man had a PSA reading of 0.40 ng/mL. His next PSA determination was 0.48 ng/mL 13 months (1.08 years) later. Calculation of PSADT based on these 2 readings is as follows:

$$\begin{aligned}\text{PSADT} &= \text{time (years)} \times \log_e(2) / [\log_e(\text{PSA2}) - \log_e(\text{PSA1})] \\ &= 1.08 \times 0.693 / [\log_e(0.48) - \log_e(0.40)] \\ &= 0.748 / [-0.734 - (-0.916)] \\ &= 4.13 \text{ years.}\end{aligned}$$

Using the first 2 PSA values for the doubling time calculation allows an assessment of the biological aggressiveness of the tumor much earlier in the course of the disease recurrence. Using this simple technique to identify patients at risk for the early development of SP allows identification of those who would be ideal candidates for clinical trials of androgen-deprivation therapy and nonhormonal treatments. Based on our results, a short PSADT (eg, 6 months) places the patient at a higher risk of developing rapid SP. Just as important, we are able to identify men who have a high probability of not developing clinical progression for extended periods of time. Such patients could be spared expensive and time-consuming diagnostic studies designed to detect metastatic disease and would be good candidates for watchful waiting. Approximately 20% of our patients who developed biochemical failure had a PSADT longer than 10 years. The SP-free survival at 10 years was 99% in this group. This group included those with fluctuating, stable, or very slowly rising PSA levels. This is an interesting group of patients; we cannot even be certain that they harbor prostate cancer because they failed to manifest clinical progression. Longer follow-up will be required to determine their outcome. Retention of benign PSA-producing tissue has been proposed as a possible explanation for a detectable but stable PSA level after radical prostatectomy.[15-18] Evaluation of these patients is beyond the scope of this study; however, these patients with a prolonged PSADT are obviously at low risk of clinical progression and can be followed up expectantly.

Although the present study provides useful information to identify risk factors for clinical progression after biochemical failure, its weaknesses are those inherent in any retrospective review. Of all 879 men who had biochemical failure, adequate data for PSADT calculations were available for only 587. This likely reflects a lack of uniform PSA monitoring. However, those with adequate PSA data appeared to be representative of the whole PSA event cohort. Also, 47% of patients in whom biochemical failure occurred received a form of adjuvant therapy after biochemical failure but before clinical SP. To approximate the natural history of clinical failure after biochemical failure, we censored follow-up at the time of adjuvant therapy. This had a minimal effect on SP-free survival, as the 5- and 10-year SP-free survival was 94% and 91%, respectively, when censored, compared with 90% and 83%, respectively, when adjuvant therapy was ignored.

For patients who have undergone radical prostatectomy, a rising PSA level suggests evidence of residual or recurrent prostate cancer. This finding provokes anxiety and fear in patients in part because a rising PSA level is often equated with impending death from metastatic disease. It is imperative that physicians interpret properly the importance of PSA elevations postoperatively and develop a treatment plan based on clinical progression data. The difficulty for clinicians lies in the fact that there is no standard of care for patients whose only manifestation of disease is a rising PSA level. When patients are asymptomatic, as is the case prior to the development of clinical progression, initiation of androgen-deprivation therapy will likely result in temporary PSA reduction along with the unwelcome adverse effects of hot flashes, anemia, accelerated osteoporosis, weight gain, lassitude, and sexual dysfunction. There are no compelling data to universally recommend immediate hormonal therapy for biochemical failure. Observation in patients with biochemical failure without any clinical evidence of disease is justified on the basis of the often protracted course of the disease and the absence of effective second-line curative therapy.

While radical prostatectomy continues to be used as an excellent therapy for clinically confined prostate cancer, biochemical failures may occur. A strong argument in favor of expectant management with delayed hormonal therapy vs early androgen-deprivation therapy cannot be made because of the lack of prospective trials required to clarify the controversy. However, today we are able to identify patients who are at high risk for early clinical progression as well as those who are unlikely to develop clinical progression. The PSADT appears to be a good predictor of time to clinical progression. High-risk patients ought to be considered for prospective clinical trials to determine efficacy of early adjuvant therapy. Conversely, patients with a PSADT of 10 years or longer can be safely observed and spared the cost and adverse effects of androgen-deprivation therapy.

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The Dynamics of Prostate Specific Antigen in Hormone Refractory Prostate Carcinoma

An Analysis of Cancer and Leukemia Group B Study 9181 of Megestrol Acetate

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BACKGROUND. Although many physicians measure serum prostate specific antigen (PSA) during the follow-up of patients with hormone refractory prostate carcinoma (HRPC), little has been done to formalize the determination of how these serial values of PSA impact on prognosis. To understand HRPC fully, make decisions about choices of treatment as well as about clinical research on treatments for HRPC patients, and design appropriate measures of PSA response, it seems that first it would be necessary to understand how these serial measures of PSA relate to survival. The purpose of this study was to determine how repeated measurements of PSA impact on the probability of imminent death for patients with HRPC.

METHODS. One hundred forty-eight men with HRPC were enrolled in Cancer and Leukemia Group B Study 9181, in which they were treated with either a low dose (160 mg/day) or a high dose (640 mg/day) of megestrol acetate (MA). Because preliminary data analysis indicated that these treatments had no effect on survival, the authors pooled the data to analyze the overall dynamics of PSA and survival during the follow-up period. The authors attempted to correlate initial and monthly PSA measurements, which were mandated by the study protocol, with the probability of death at any time during follow-up. For statistical analysis, the Cox proportional hazards model and the general linear model were used. In addition to the level of PSA, the authors used the relative velocity of PSA, which was defined as $(dy/dt)/y$, with "y" symbolizing serum PSA and "t" symbolizing time.

RESULTS. Both log(PSA) and the average relative velocity of PSA (rva) were significantly correlated with survival time ($P = 0.0001$ and $P = 0.0008$, respectively), and the analysis performed with the Cox proportional hazards model yielded the following formula for a PSA hazard score:

$$\text{PSA hazard score} = 0.251*(\log(\text{PSA}) - \text{mean } \log(\text{PSA})) + 24.5*(\text{rva} - \text{mean rva})$$

This hazard score tended to be higher for patients who were about to die. For example, there was a close correlation between the hazard score and the probability of death as the next observed event. Furthermore, the hazard score provided a dynamic measure of how PSA was affected by treatment.

CONCLUSIONS. The average relative velocity of PSA has been identified by the authors as a new measure of the dynamics of PSA in HRPC. It can be determined from sequential values of PSA. This average, together with the log(PSA), are significantly related to the probability of imminent death. *Cancer* 1998;83:1989–94.
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KEYWORDS: prostate specific antigen, kinetics, prostate carcinoma, megestrol acetate.

In 1993, Andriole described prostate specific antigen (PSA) as "the most useful tumor marker,"[1] and it is clear that PSA has played important roles in the diagnosis and staging of prostate carcinoma, in the selection of treatments, and as a measure of response to treatments. Nevertheless, it also seems that there is more to learn about the relations between PSA and tumor burden, treatment response, and survival. For example, one of the most difficult issues about PSA is that, at any time after treatment, its connection to further prognosis makes it a measure of both response as well as prognosis, that is, response to previous events and prognosis for future events. In fact, it is its connection to eventual outcome that makes PSA measures after treatment attractive surrogates for survival outcome. Unfortunately, as a measure of either response or prognosis, PSA is a moving target. Serial measures of PSA in time describe a dynamic process rather than a static one, and this has caused uncertainty about how and when to define a PSA-related response. In hormone refractory prostate carcinoma (HRPC), Scher et al.[2] recently suggested that PSA has been used inconsistently as a measure of response. They wrote, "there are no standard methods of reporting outcomes; more importantly, none of the 'criteria' have been validated in the Phase III setting using the *definitive end point of survival* (emphasis added)."[2] Following the spirit of this comment, we sought to study how serial records of PSA relate to instantaneous hazard for death. We used serial PSA data from 148 men with HRPC who participated in a randomized Cancer and Leukemia Group B (CALGB) Phase II trial (CALGB 9181) designed to study the effect of dose of megestrol acetate (MA) on survival, and 137 men were evaluable. We found that, at any time during follow-up, both the level of PSA and the average relative velocity of PSA related to the hazard of death. Together, these two yield a PSA hazard score that may provide a good measure of PSA-related response.

PATIENTS AND METHODS

One hundred forty-eight men with histologically documented carcinoma of the prostate, with metastatic tumor, and with progression after standard hormonal therapy were entered on CALGB randomized Phase II protocol 9181 comparing low dose MA (160 mg/day) with high dose MA (640 mg/day). Written, informed consent was obtained from all the patients, and the study was approved by all CALGB participating member institutional review boards. The protocol required that blood be drawn and assayed for PSA within the 2-week period before entry and at 4-week intervals thereafter. In addition, 40 men had more than one PSA value recorded on the CALGB flow sheets prior to entry. At the time of analysis, 23 patients were alive at a median of 2 years follow-up, and the remaining patients had died. Of the initial 148 patients, 137 were evaluable for follow-up and for key variables of the study. PSA was measured in each patient's institutional laboratory. When noted, the methods commonly were either the assay by Hybritech or by Abbott, but the method was often ommitted from the record. Their median number of PSA values was 6, with a range from 1 to 39, and the median number of days between PSA measurements was 29 days. This report deals with the dynamics of PSA in these 137 patients.

PSA Relative Velocity

For brevity, if we symbolize serum PSA as "y" and time as "t," then the velocity of PSA is dy/dt, and relative velocity is defined as

$$\text{Relative velocity} = \frac{dy/dt}{y}, \quad (1)$$

and, because this also can be recognized as the derivative of the natural logarithm of y with respect to time, a way to estimate the relative velocity during any time interval t1 to t2 is

$$\text{Relative velocity} = \frac{\log(y2) - \log(y1)}{t2 - t1} = \frac{\log(y2/y1)}{t2 - t1}, \quad (2)$$

where y1 denotes the value of PSA at t1, and y2 denotes the value at t2. Throughout this paper "log" refers to the natural logarithm, that is, logarithm to the base of "e," which is approximately 2.72. Thus, the relative velocity for any time interval is just the slope of the log(PSA) versus time curve at that interval. The average relative velocity at any time is just the average of these individual relative velocities up to that time.

Statistical Methods

To model survival time and hazard function, we used the Cox proportional hazard model,[3] as manifest in the Coxph program of the S-PLUS software,[4] and, to adapt the analysis to the serial values of PSA as a time-dependent variable, we used the "interval censored" or "counting process" approach.[5–7] Time intervals for patients were defined by the time points for sequential pairs of PSA values, and only the prognostic variables available at the beginning of the interval were used in the Cox modeling. For any time of death, surviving patients with multiple PSA values contributed just once to the hazard denominator, and their

PSA changed with each time. Because the Cox model assumes a proportional hazard function, the hazard at any time h(t) can be related to a baseline hazard ho(t) by the following equation:

$$h(t) = ho(t)*\exp(b1*x1(t) + b2*x2(t) + \ldots), \quad (3)$$

with the x1(t), x2(t), . . . being prognostic variables that change with time. In this paper, we will refer to the sum "b1*x1(t) + b2*x2(t)" as a "hazard score" for a two-variable model. Because this hazard score relates to the relative hazard through Equation 3, it closely relates to survival probability.[3] In our use, it will also provide a composite measure of both static and dynamic aspects of PSA, and we will use it to study how dosage of MA affects the dynamics of PSA during time of follow-up. For this analysis, we use the general linear model.[8]

RESULTS

Figure 1 shows the temporal pattern of log(PSA) for four representative patients in our study. All four demonstrate fluctuations in PSA level as well as a rising trend in PSA.

Table 1 shows the results of a Cox proportional hazard analysis for survival time using two PSA time-dependent variables: log(PSA) and the average relative velocity (rva). For these two variables, the coefficients are the "b1" and "b2" of Equation 3, the standard errors refer to the expected variance in the estimates of these coefficients, and the *P* values indicate their level of individual significance for association with survival time. These results show that both log(PSA) and the average relative velocity relate significantly to survival time, as measured from the current follow-up time until the next event. Using the coefficients, we can write the PSA hazard score as

$$\text{PSA hazard score} = 0.251*(\log(\text{PSA}(t)) - \text{mean } \log(\text{PSA})) + 24.5*(\text{rva}(t) - \text{mean rva}). \quad (4)$$

For our patients, the mean log(PSA) was 4.93, and the mean rva was 0.00624.

The "(t)" in the equation reminds us that PSA hazard score is a function of time of follow-up, because both PSA(t) and rva(t) change with time. For example, Figure 2 shows the calculated hazard scores over time of follow-up for the same four patients illustrated in Figure 1. Patient 52,897's hazard scores rose steadily throughout the study, that is, by this measure, he showed no evidence of a response; and he died on Day 662. On the other hand, patients 53,304, 53,932, and 54,220 showed hazard scores that dropped and mostly remained below "0." These values, therefore, suggested a response. Eventually, patients 53,304 and 54,220 had rising hazard scores, although both were living at their last contacts (Days 982 and 901, respectively). Patient 53,932 died at Day 1015, but, unfortunately, our measurements stopped just after Day 500, so that we do not know how his hazard score changed in the last 500 days. Figure 3 provides a further overview of the importance of the PSA hazard score to survival time. Here, the observed probability of death as the next event is plotted against the PSA hazard score, and the curved line gives the result. We see that, as the hazard score rises above "0," the probability of death as the next observed event rises above 0.2 and eventually exceeds 0.4. This relation is strictly a probabilistic one, because, at each level of hazard score, there are those who survive (Fig. 3, dots at the bottom of the plot) and those who die (Fig. 3, dots at the top of the plot). Thus, the PSA hazard score predicts for a population or for an average patient, but not for an individual. On the other hand, because it relates to survival for a population of patients, it may be a suitable measure for PSA-related outcome of a study such as CALGB 9181.

Change in PSA Hazard Score with Treatment

Finally, we examined how dosage of MA affected the PSA hazard score over the course of follow-up. Rather than selecting a particular time after treatment to test for a treatment effect, we examined the entire time of follow-up, that is, we looked for overall trends throughout follow-up. Table 2 shows the results of a general linear model analysis of how the PSA hazard score changed with time and treatment. First, we found that the score had a parabolic relation with time, that is, it initially dropped and later rose. The parabola is reflected by the negative coefficient for the first power of "time" and the positive coefficient of the second power of "time," together with their significant *P* values. Because both powers of time were significant, thorough testing for a dosage effect over the entire time of follow-up required three dosage variables. The first, "dose," was to test for an overall effect of dosage of MA regardless of time or a dosage effect at the beginning of the study when time was "0." The second ("dose × time") and third ("dose × time^2") were required to test for an effect of dosage of MA on how the PSA hazard score changed with time. The results show that higher dose of MA was associated with a borderline elevation of PSA hazard score at the beginning of the study ($P = 0.059$) but with a slightly lower slope with time ($P = 0.024$). The magnitude of the slope coefficients indicate that high dose resulted in a 25% decrement in slope, that is, hazard scores fell faster with time in those patients on high dose. These

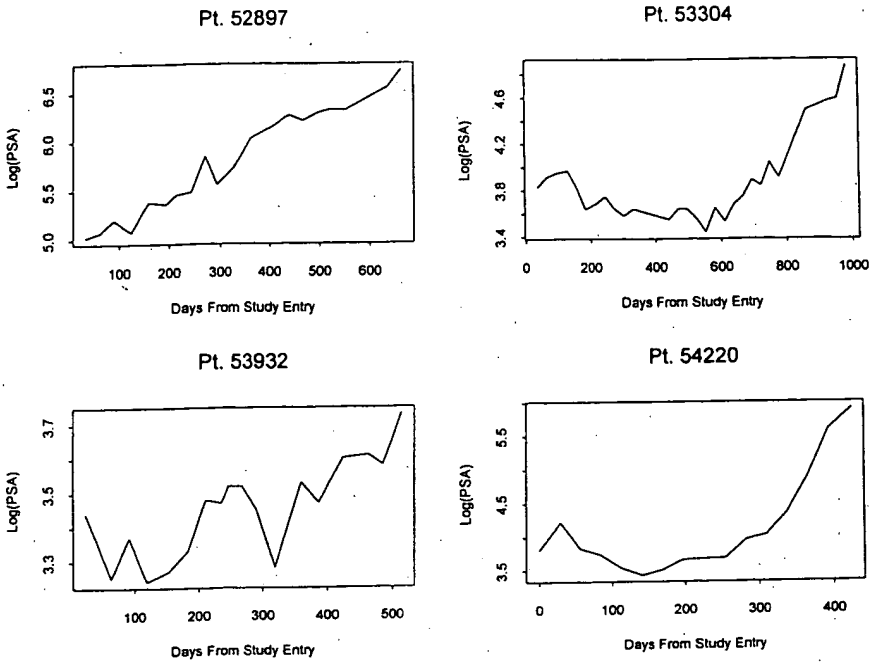

FIGURE 1 Composite of four plots showing log(PSA) versus time of follow-up for four patients in the study. Log, natural logarithm; PSA, prostate specific antigen.

TABLE 1
PSA-Based Survival Model[9]

Measure	Log(PSA)	rva
Coefficient	0.221	24.5
Standard error	0.057	7.32
P value	0.0001	0.0008
Mean value	4.93	0.00624

Log: natural logarithm; PSA: prostate specific antigen; rva: average relative velocity of PSA.
[9] No. = 137 patients; model likelihood ratio = 28.7.

differences are best seen in Figure 4, which shows the plot of the expected average hazard scores over time for the two dosages of MA. We see from the plot that the higher dose of MA gives a higher level of score at the beginning; it produces a slightly steeper fall, and there is a slower rate of rise in the later times of follow-up that, by itself, was not significant ($P = 0.66$).

DISCUSSION

Although we often use PSA as a measure of response to treatment, the results of this study remind us that survival time is the real measure of response and that, during follow-up, both PSA and probability of dying are moving targets. As a changing variable, PSA clearly is connected to survival probability, but our results now suggest that there is more complexity in the way PSA connects to survival hazard than we might have guessed before this study. We must consider not only specific values of PSA but also a kinetic measure, such as the average relative velocity. At any time, the hazard for death is a continuously changing function of log(PSA) plus the average of a series of relative velocities of PSA tallied over a period of time. The success of our time-dependent model also suggests that we cannot simply select a specific time or specific value of PSA for response. For example, the fluctuations of raw PSA as well as of the PSA hazard score in patients such as those illustrated in Figures 1 and 2 emphasize the difficulty of picking a time point for defining a PSA-related response to treatment—any such time point seems arbitrary. Instead, we must consider measures that use more of the PSA time curve, and possibilities include the area under the PSA time curve or a general linear model that relates PSA or a PSA hazard score to both treatment and time, as shown in Table 2 and Figure 4.

Regarding the way treatment affects PSA, the issues are complex. First, because all of our patients were treated with MA, it is possible that the form or coefficients of the hazard score in Equation 4 may reflect an effect of MA. For example, the negative coefficient for "time" in the general linear model of Table 2 indicates that there was initially a downward trend in hazard score for our average patient. This trend could have been due to MA. It also could have been due to the early deaths of 32 patients who had relatively high hazard scores at the beginning of the

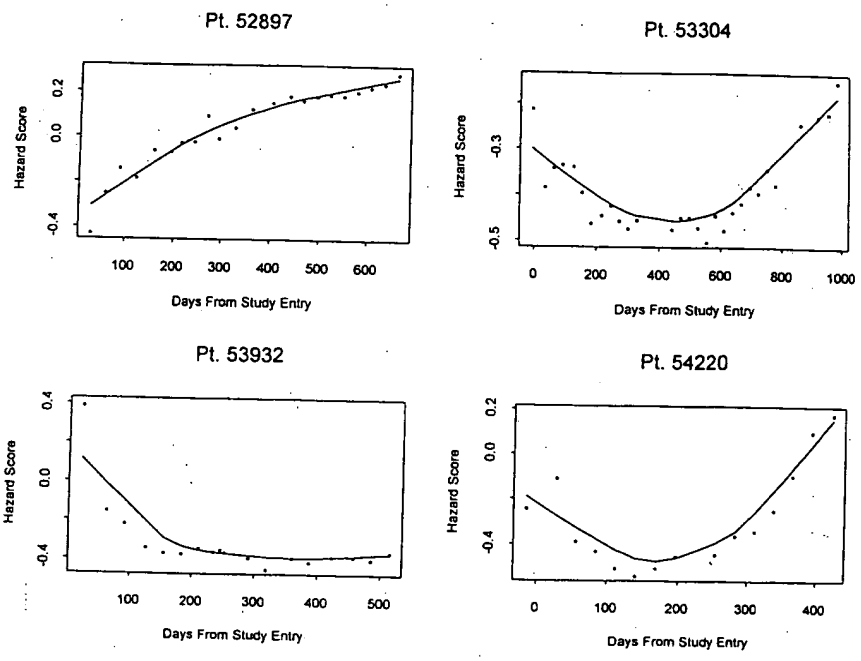

FIGURE 2 Composite of four plots showing calculated hazard score versus time of follow-up for the four patients from Figure 1. The lines are provided by the lowess function in S-PLUS,[4,6] and they demonstrate the smoothed trend in the data.

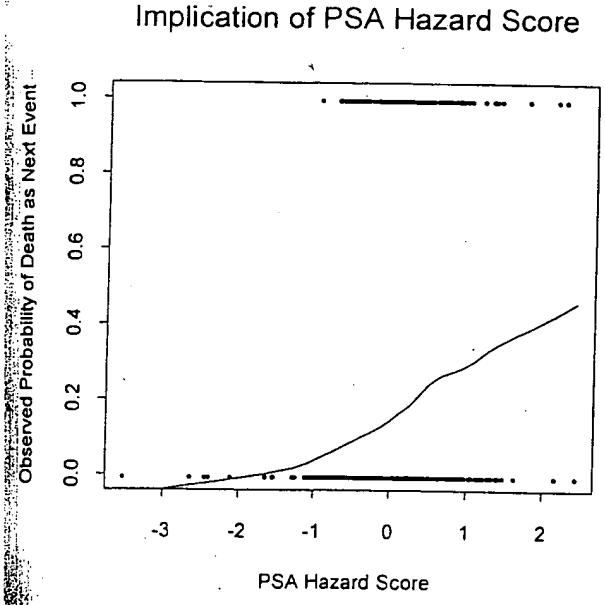

FIGURE 3 Plot of the observed probability of death as the next event versus a clinic visit (i.e., no death) on the vertical axis versus the calculated PSA hazard score on the horizontal axis. The curved line provides the result and was obtained with the lowess function in S-PLUS. The dots at the top indicate observed deaths, and the dots at the bottom indicate observed clinic visits (i.e., no deaths).

study, because, after these patients died, the hazard scores reflected only those who were to survive longer. Because we had no group of untreated patients in our study, we cannot choose between these two interpretations or even some combination. Despite the lack of an untreated group of patients, our study demonstrates a new way to identify treatment effects. If treatment affects survival without changing the relation between survival and PSA, then the Cox model may not show a significant treatment effect when PSA or other prognostic variables are included in the model. An alternative to this traditional approach is to find first how survival relates to prognostic variables, such as PSA, and then test how treatment affects the optimized hazard score.

In constructing the PSA hazard score, we emphasized PSA because it was the only variable consistently included in our data base during follow-up and because PSA has been the one variable emphasized for measuring response to treatment. Nevertheless, there

TABLE 2
General Linear Model of PSA Hazard Score

Variable	Coefficient	SE	F	*P* Value
Intercept	0.063	0.049	—	—
Time	-1.15×10^{-3}	4.56×10^{-4}	18.1	2.3×10^{-5}
Time^2	1.54×10^{-6}	7.50×10^{-7}	7.3	0.0068
Dose of MA	0.138	0.0667	3.6	0.059
Dose × time	-2.87×10^{-4}	6.13×10^{-4}	5.1	0.024
Dose × time^2	-4.05×10^{-7}	9.24×10^{-7}	0.2	0.66

PSA: prostate specific antigen; SE: standard error of the estimate of coefficient; F: F statistic; MA: megestrol acetate.

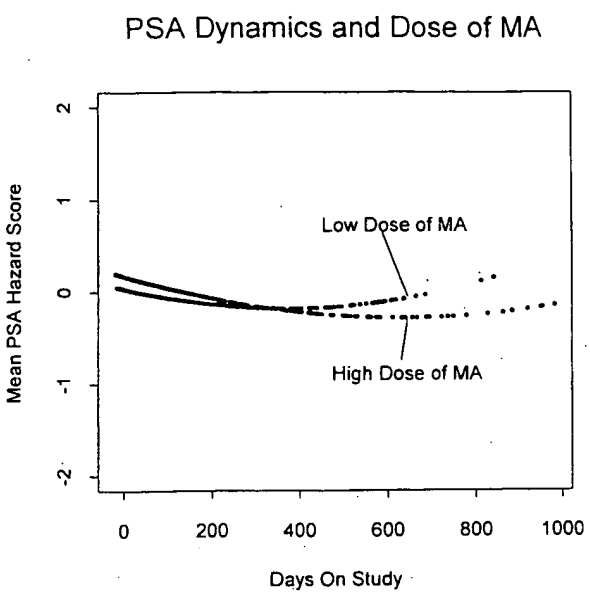

FIGURE 4 Plot of mean of PSA hazard scores versus time of follow-up for those on low dose and high dose megestrol acetate (MA).

may be other variables that are important to survival and are measured repeatedly, like PSA. For example, hemoglobin, weight, and performance status come to mind. Thus, in the future, what may relate more closely to survival could be a time-dependent hazard score that combines PSA-related variables with non-PSA variables. The closer hazard score is connected to survival, the better it should measure response.

Finally, we emphasize that it may be important to examine PSA as well as the relative velocity of PSA at the beginning of studies of HRPC. For example, the combination of these two variables may identify patients with high probability for dying before they complete the treatment. To make treatment groups comparable and eliminate bias at the onset of study, one should strive to see that both groups have the same initial hazard score. This was not true for our study, although we had no way of knowing this until the analysis was done.

In summary, we have learned that repeated measures of PSA are prognostic in men with HRPC and yield a useful kinetic measure, the average relative velocity of PSA. This average, as a PSA variable, has three key features. It measures change in PSA with respect to time. Because it is constructed as a ratio (see Eq. 2), some PSA assay variation is reduced in the factor. Finally, as an average it is a measure of overall trend. Thus, despite variations due to assay or to biology in our raw data, the logarithm of PSA and the average relative velocity from these repeated measures were significantly associated with survival. The resulting PSA hazard score helped us recognize a small treatment effect in our study, and it could have identified an initial bias in our study had we known of it. Because our study was not designed for our analysis, some PSA values were missing, especially as the patients neared death. In other studies, the exact form and coefficients of the hazard score may vary, so that we consider the details of our results to be preliminary. However, as a general approach, we believe that use of the level of PSA, the average relative velocity of PSA, and the hazard score shows promise for prognostication in HRPC as well as for measuring response to treatment. We conclude that monthly PSA samples spaced throughout and after therapy in HRPC provide important information in this disease, and future CALGB studies of HRPC will include these measurements.

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